A STUDY OF AUDITORY BRAINSTEM RESPONSE AND OXIDATIVE STRESS IN INDIVIDUALS WITH VITILIGO

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APRIL – 2016

CERTIFICATE

This is to certify that this dissertation entitled **"A STUDY OF AUDITORY BRAINSTEM RESPONSE AND OXIDATIVE STRESS IN INDIVIDUALS WITH VITILIGO"** by the Post Graduate **Dr. K. LATHA** for **M.D. (PHYSIOLOGY), BRANCH – V** is a bonafide record of the research done by her during the period of study (2013 – 2016) in Stanley Medical College, Chennai – 600 001.

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DECLARATION

I, Dr. K. LATHA, solemnly declare that this dissertation entitled, **"A STUDY OF AUDITORY BRAINSTEM RESPONSE AND OXIDATIVE STRESS IN INDIVIDUALS WITH VITILIGO"** is a bonafide and genuine research work done by me at Govt. Stanley Medical College and Hospital during 2013 – 2016 under the guidance and supervision of **Dr. K. BALASUBRAMANIAN, M.D.,** Professor and Head, Department of Physiology, Stanley Medical College, Chennai – 600 001.

The dissertation is submitted to the The Tamilnadu, Dr. M.G.R. Medical University, towards partial fulfilment of the requirements for the award of **M.D.Degree (BRANCH – V) in Physiology.**

Place : Chennai – 600 001

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ABBREVIATIONS

SON	Superior olivary nucleus
ABR	Auditory Brainstem Response
ADC	Analog – to – digital converter
BAEP	Brainstem Auditory Evoked Potential
BERA	Brainstem Evoked Response Audiometry
CAT	Catalase
GPx	Glutathione peroxidase
HLA	Human Leukocyte Antigen
IPL	Inter Peak Latency
NO	Nitric oxide
NSV	Non-segmental vitiligo
PUVA	Psoralen compounds and ultraviolet A
ROS	Reactive Oxygen Species
SNR	Signal – to – noise ratio
SOD	Superoxide dismutase

A STUDY OF AUDITORY BRAINSTEM RESPONSE AND OXIDATIVE STRESS IN INDIVIDUALS WITH VITILIGO

ABSRACT

Introduction: Vitiligo is an idiopathic, acquired, hypomelanotic, systemic disorder, affecting the whole pigmentary system including the stria vascularis of the inner ear, characterized by depigmented patches of different sizes and shapes in the skin resulting from the loss of functional melanocytes and melanin from the epidermis. Vitiligo occurs worldwide with a prevalence of 0.1% to 0.2 %. In India, the prevalence is 3% to 4%. Vitiligo commonly begins in childhood or young adulthood, with peak onset of age being 10 to 30 years, but it may occur at any age. All races are affected. Both gender are equally affected. Approximately 20% of individuals with vitiligo have atleast one first degree relative with vitiligo. The etiology of vitiligo is still not known, but several theories have been proposed to explain the melanocyte destruction like genetic, neural, cytotoxic and auto-immune theories. Oxidative stress has been implemented in the pathophysiology of vitiligo. During oxidative stress, the molecular oxygen is reduced to form super-oxide radicals. Further, superoxide radicals dismutate to hydrogen peroxide, either spontaneously or by the action of superoxide dismutase. **Aim of the study** was (1) To evaluate the conduction in the auditory pathway in vitiligo subjects. (2) To study the role of oxidative stress in vitiligo subjects. **Objectives of the study** were (1) To evaluate the audiological abnormalities using Brainstem Evoked Auditory Response as a tool in individuals with vitiligo.(2) To study the role of oxidative stress by assay of superoxide dismutase enzyme level in individuals with vitiligo. (3) To do a comparative study in a healthy control group. **Materials and methods:**Ethical approval from Institutional Ethical Committee, Stanley Medical College, Chennai-1 was obtained for the study. In this study, Brainstem Auditory Evoked Potential (BAEP) was recorded in 40 individuals affected by vitiligo of various dermatomes and distribution, recruited from the Department of Dermatology

and Department of Cosmetology, Stanley Medical College, Chennai-1, who fulfilled the selection criteria. 40 age and gender matched healthy individuals were taken as controls. BAEP recording was done in the Neurophysiology Laboratory of Research Wing, Department of Physiology, Government Stanley Medical College, using Neuro Perfect Plus – Medicaid Polyrite Hardware – Solokrafts Industries India. Brainstem Auditory Evoked Potential of both the ears were tested. Absolute wave and Inter Peak Latencies were measured. Under strict aseptic precautions, venous blood samples were collected from both the vitiligo individuals and controls, for estimation of superoxide dismutase (SOD) enzyme level using ELISA kit in the laboratory. **Results:** A significant increase in the Absolute wave latency III ($P < 0.01$) of left ear was found. There was prolonged Absolute wave latency III of right ear ($P = 0.001$) and prolonged Inter Peak Latency I-III ($P < 0.001$) of both the ears and they were highly significant when compared with that of the controls. A decrease in the serum superoxide dismutase level was noted when compared with that of the controls, but it was not statistically significant. **Conclusion:** Audiological changes in vitiligo subjects confirm that the vitiligo represents a systemic disease with widespread involvement of body melanocytes. The reduced levels or reduced activities of these melanocytes or both may be the cause for audiological abnormalities. Also, the antioxidant level was decreased in vitiligo subjects when compared with that of the controls suggesting a state of oxidative stress in them.

Keywords: Brainstem Evoked Response Audiometry, Brainstem Auditory Evoked Potential, Vitiligo, Superoxide dismutase enzyme, Oxidative stress.

1. INTRODUCTION

Vitiligo is an idiopathic, acquired, hypomelanotic, systemic disorder, affecting the whole pigmentary system including the stria vascularis of inner ear, characterized by depigmented patches of different sizes and shapes in the skin resulting from the loss of functional melanocytes and melanin from the epidermis. Vitiligo occurs worldwide with a prevalence of 0.1% to 0.2 %. In India, the prevalence is 3% to 4%. Vitiligo commonly begins in childhood or young adulthood, with peak onset of age being 10 to 30 years, but it may occur at any age. All races are affected. Both gender are equally affected. Approximately 20% of individuals with vitiligo have atleast one first degree relative with vitiligo.

The etiology of vitiligo is still not known but several theories have been proposed to explain the melanocyte destruction like genetic, neural, cytotoxic and autoimmune theories. Oxidative stress is thought to be the initial pathogenic event in melanocyte destruction [72,73]. Normally free radicals such as superoxide, hydrogen peroxide and nitric oxide are formed during several physiological and pathological processes [94]. They are continuously scavenged by antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase etc. During oxidative stress, the molecular oxygen is reduced to form superoxide radicals. Further, superoxide radicals dismutate to hydrogen peroxide either spontaneously or by the action of superoxide dismutase[70,71]. The enzyme catalase converts H_2O_2 to O_2 and water. In case

of oxidative stress, to scavenge or counteract the increased levels of superoxide anions (O_2^-), there is an increase in the levels of SOD, an antioxidant enzyme, whereas the catalase levels are decreased [56]. The formed H_2O_2 readily crosses the cell membranes and can cause much of the damage.

Several studies suggest that the accumulation of free radicals which are toxic to melanocytes may lead to their destruction. The inherent capacity of cells to withstand the oxidative stress is due to their ability to repair oxidatively modified biomolecules, their antioxidant ability and their capacity to sustain metabolic requirements by deriving energy from alternate pathways.

Several studies have been done in vitiligo subjects on various biomarkers such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and nitric oxide (NO). Different results were reported about the levels of these biomarkers. A few studies have reported an increase in the serum SOD levels, few others have observed a reduction in the SOD levels while some studies have observed no change in the blood levels of SOD. Hence, this study was undertaken to assess the oxidative stress in vitiligo subjects by estimating the levels of SOD in the blood. This will enable us to suggest the use of antioxidants as an adjuvant in the management of vitiligo.

The mechanism destroying the melanocyte in the skin could also affect other melanocyte organs[5]. Several ocular[6] and audiological abnormalities of hearing[7-13] and brainstem responses(BAERs)[10-12] have been reported. The purpose of this study was to detect the subclinical audiological changes in patients with vitiligo. These patients are usually asymptomatic. Hence, physiological testing of the function of the eighth cranial nerve may provide sensitive, quantitative measures of mild or early damage to its peripheral or central components [46]. Hence, this study was undertaken in subjects with vitiligo with the aim

1. To evaluate the audiological abnormalities using Brainstem Evoked Response Audiometry as a tool .

2. To assess the oxidative stress by assay of serum superoxide dismutase enzyme level.

2. REVIEW OF LITERATURE

Vitiligo denotes an acquired, idiopathic, usually progressive, circumscribed achromic macules. It is often associated with leukotrichia. Histologically it is characterised by degeneration and disappearance of melanocytes in the involved skin and not infrequently in the pigment epithelium of the inner ear and pigment epithelium of eyes[1].

2.1.1 CLINICAL PRESENTATION

a) Focal vitligo : Solitary macule or a few scattered macules in one area most commonly in the trigeminal area.

B) Segmental vitiligo : Unilateral macules in a dermatomal or quasi – dermatomal distribution.

c) Acrofacial vitiligo : Depigmentation of distal fingers , toes and periorificial areas.

d) Mucosal vitiligo : Involvement of mucosal membrane sites only.

e) Generalized vitiligo : Also called vitiligo vulgaris. It is the most common pattern. Depigmented patches are widely and usually symmetrically distributed.

f) Universal vitiligo : Depigmented patches over most of the body often associated with multiple endocrinopathy syndrome.

Diagnosis of vitiligo is primarily based on clinical examination. Since it is of autoimmune in origin , investigations may be done to rule out other endocrine disorders. The prognosis and course of vitiligo are unpredictable. Initial clinical subtype does not predict future anatomical sites of involvement or activity of the disease[2].

2.1.2 EPIDEMIOLOGY

Vitiligo affects all the races the world over. Higher incidence has been reported in India and Mexico. In India, it is roughly estimated to be 3% - 4%. Prevalence is high in the states of Gujarat and Rajasthan in India. Several other studies have reported the incidence of 0.1% - 2 % in different parts of the globe. Vitiligo commonly begins in childhood or young adulthood . The peak onset of age being 10 to 30 years, but it can occur at any age [1,20].

All races are affected. The incidence among the gender follows the general population pattern without any particular predilection. Some reports of female preponderance probably reflect their great concern for cosmetic disfigurement and related to the social and marital problems.

Vitiligo mostly affects people with skin types III and IV. A higher incidence has been reported in patients with atopic dermatitis.

Onset of unilateral dermatomal type is usually in childhood within 10 years of age. Bilateral non- dermatomal lesions begin in the second to fourth decades of life. Onset at the extremes of age is less common[1,20].

Fitzpatrick Classification Scale

Updated in November 25, 2014. The Fitzpatrick Classification Scale was developed in 1975 by Harvard Medical School dermatologist, Thomas Fitzpatrick, MD, PhD. It is used by many practitioners to determine the way one will respond or react to facial treatments and his risk of getting skin cancer.

This scale classifies a person's complexion and their tolerance of sunlight.

Table 1

Skin Type	**Skin Color**	**Characteristics**
I	White; very fair; red or blond hair; blue eyes; freckles	Always burns, never tans
II	White; fair; red or blond hair; blue, hazel, or green eyes	Usually burns, tans with difficulty
III	Cream white; fair with any eye or hair colour; very common	Sometimes mild burn, gradually tans
IV	Brown; typical Mediterranean caucasian skin	Rarely burns, tans with ease
V	Dark Brown; mid-eastern skin types	very rarely burns, tans very easily
VI	Black	Never burns, tans very easily

2.1.3 ETIOPATHOGENESIS OF VITILIGO

Vitiligo is a multifactorial polygenic disorder with complex pathogenesis. Although several theories have explained the loss of epidermal melanocytes in vitiligo, the precise cause remains unknown[2].

The theories include autoimmune, cytotoxic, biochemical, oxidant – antioxidant and neural mechanisms for destruction of melanocytes[21].

Various precipitating / aggravating factors also play a role in etio-pathogenesis of vitiligo like trauma – physical or emotional [3].

Autoimmune theory :

This theory is based on the association of vitiligo with a number of autoimmune disorders. Organ - specific autoantibodies to thyroid, gastric parietal cells and adrenal tissue are found in the serum of individuals with vitiligo than in general population[22,23]. A complement fixing antibody to melanocytes has been found in the serum of several patients, who in addition to vitiligo had alopecia areata, mucocutaneous candidiasis and multiple endocrine insufficiencies[22].

Antibodies to human melanocytes have been detected using a specific immunoprecipitation assay[24,25] and they have a cytolytic effect[26]. T-cell profiles are abnormal in vitiligo with a decrease in T-helper cells[27-29].

Neurogenic theory :

This theory suggests that a compound is released at peripheral nerve endings in the skin that may inhibit melanogenesis.

Recent studies on neuropeptide and neuronal markers in vitiligo suggest that neuropeptide Y may have a role[30]. Additionally, depigmented areas showed some abnormal autonomic function such as increased adrenergic tone and increased concentration of catecholamines[31].

Segmental vitiligo which occurs frequently in a dermatomal pattern, supports this hypothesis. Ito and others observed the close interaction between melanin synthesis and the autonomic nervous system. This also includes the occurrence of vitiligo in neurologically compromised skin, following viral encephalitis, following peripheral nerve injury, among emotionally hyperstressed and psychiatric patients[31,32].

Self-destruct theory of Lerner :

This suggests that melanocytes destroy themselves due to a defect in natural protective mechanism, which removes some of the pre-melanogenesis metabolites. This hypothesis is based on experimental studies of cutaneous depigmentation by chemical compounds that have selective lethal effect on functional melanocytes[33].

Oxidative stress theory :

Oxidative stress is reported to play a role in progress of vitiligo. During oxidative stress, molecular oxygen is reduced to form superoxide radicals. Further, superoxide radicals dismutate to hydrogen peroxide either spontaneously or by the action of superoxide dismutase.

In epidermal cells, hydrogen peroxide levels increase due to oxidative stress from environmental trauma such as Ultra Violet radiation B (290 – 320 nm). Moreover, there is a basal level of oxidants in cells that are the by-products of normal endogenous metabolic processes.

Although a system of enzymatic and non-enzymatic antioxidants provide protection against Reactive Oxygen Species (ROS), an imbalance between oxidants and antioxidants can lead to disruption of cellular functions[34].

Antioxidant deficiency theory :

According to this hypothesis, there is a breakdown in the antioxidant defence of the epidermis leading to melanocyte toxicity. An abnormally high catecholamine discharge in the epidermis and dermis could lead to skin ischaemia and subsequent overproduction of Reactive Oxygen Species (ROS).This theory is supported by the observation of low levels of catalase activity in the epidermis of vitiligo subjects. This oxidative stress could lead to

the accumulation in the skin of high concentrations of 6- and 7- biopterins, which inhibit tyrosinase activity and are extremely cytotoxic to melanocytes[20].

The antioxidant imbalance has also been confirmed in peripheral blood mononuclear cells of vitiligo subjects. It was correlated to raise intracellular production of ROS and impairment of mitochondria[35]. These findings support the concept of a possible systemic oxidative stress in vitiligo.

Melanocyte Growth Factor reduction hypothesis :

The normal population density of melanocytes in the skin is possibly regulated by a growth factor originating from keratinocytes, fibroblasts and various other tissues.

It has been proposed that depigmentation in vitiligo might be due to reduced local and circulating levels of growth factor, which is necessary for the normal proliferation and maintenance of melanocytes[20].

Genetic factors :

Some vitiligo individuals give a family history of vitiligo, which probably indicates the role of genetic factors in the pathogenesis. Earlier, vitiligo was thought to be seldom inherited. At present, the consensus is that the familial incidence is between 20% and 30%.Inheritance was thought to be autosomal dominant with variable expression and incomplete penetrance.

The possibility of an autosomal recessive or its polygenic inheritance with somatic expression decided by precipitating factors has also been suggested. Recent observations suggest a polygenic multifactorial inheritance and a role of acquired factors for its chemical expression.

No Human Leukocyte Antigen (HLA) is established for vitiligo, although associations with HLA-DR4 in black coloured people, HLA-B13 in Moroccan Jews and HLA-BW35 in Yemenite Jews with vitiligo have been reported (20). Vitiligo has been reported in monozygotic twins also[36].

Melanocytorrhagy in vitiligo:

Dendrites of melanocytes help in transfer of melanosomes to the surrounding keratinocytes and also help in their adherence to basal membrane. Cultured vitiligo melanocytes show stubby dendrites. Cultured normal melanocytes show loss of dendrites and sometimes melanocyte detachment on adding hydrogen peroxide.

New theory of melanocytorrhagy proposes that Non-segmental vitiligo (NSV) is a primary melanocytorrhagic disorder with altered melanocyte response to friction and possibly other types of stress including their detachment and subsequent transepidermal loss. Loss of melanocytes is not balanced by influx of melanocytes from the follicular reservoir, where melanocyte stem cells are probably situated [37].

Convergence theory :

Researchers have come to a conclusion that genetic influences have a role in causing vitiligo in addition to stress, accumulation of toxic compounds, infection, autoimmunity, mutation and melanocyte proliferation[38].

2.1.4 CLINICAL FEATURES

Vitiligo can begin at any age, but in 50% of cases it develops before the age of 20 years. The condition is gradually progressive, sometimes extending rapidly over a period of several months and then remaining quiescent for many years. Hypomelanotic macules are usually first noted on the sun exposed areas of skin, on the face or on the dorsa of hands. Damage to the normal skin frequently results in an area of depigmentation – Koebner phenomenon. Areas subjected to frequent trauma are likely to be affected, for example the dorsa of hands, feet, elbows, knees and ankles.

The distribution of lesions is usually symmetrical, although sometimes it is unilateral and may have a dermatomal arrangement. Rarely there is complete vitiligo, although a few pigmented areas always remain.

A typical lesion is a well-defined depigmented macule, which often shows a variable number of depigmented hairs and without any change in the skin texture. In many cases, the margin is hyperpigmented. Sometimes the depigmented area is surrounded by a comparatively hypopigmented zone and is

seen to be separated from the normal skin by a thin hyperpigmented rim (trichrome vitiligo).

Quadrichrome vitiligo shows marginal perifollicular hyper-pigmentation characteristic of repigmenting vitiligo[20].

Pentachrome vitiligo with five shades of colour has been described. Blue vitiligo is vitiligo macules occurring in the sites of post-inflammatory hypermelanosis[39].

In addition to premature greyness of the hair, uveitis also rarely occurs[40]. Careful examination of ocular fundus may show abnormalities[41]. Also, there has been reports of increased incidence of deafness[42].

2.1.5 **CLASSIFICATION OF VITILIGO**

Vitiligo is classified based on the distribution, extension and number of hypopigmented lesions into localized and generalized types[43]. Depending upon the progression of the lesion, it can be classified into stable and unstable vitiligo[44].

A. I. Localized vitiligo:

1) Focal vitiligo :

Usually a solitary macule or a few scattered macules in one area (focal) and non-dermatomal distribution.

2) Segmental vitiligo:

Unilateral macules in a dermatomal or quasi-dermatomal distribution. This type of vitiligo has an early onset of age. It is not associated with thyroid disease or other autoimmune diseases.

3) Mucosal type:

Involvement of mucous membrane sites only.

A. II. Generalized vitiligo:

1) Acrofacial vitligo:

Depigmentation of the distal parts of limbs (hands and feet), face and periorificial areas.

2) Vitiligo vulgaris:

It is the most common pattern. Depigmented patches are widely and usually symmetrically distributed.

3) Vitiligo universalis:

In some cases, almost the entire body surface is achromic, with only a few small islets of normally coloured or hyperpigmented skin.

B. I. Stable vitiligo:

No progression of existing disease or appearance of new lesions in the last six months.

B. II. Unstable vitiligo

The appearance of new lesions and / or increase in the size of existing lesions in the last six months and/or duration of disease less than six months.

2.1.6 COURSE OF VITILIGO

The course of the disease is unpredictable and uncertain, most of them showing tendency towards slow progression. Lesions in the pseudo- segmental type remains static for an indefinite period after a certain degree of regional extension.

In vitiligo vulgaris, lesions develop on different areas in succession with varying rapidity. In some, extension of individual lesions and development of new lesions at different sites occur in episodic bouts with the intervening quiescent period varying from weeks to years. Many lesions remain static for an indefinite period or show some degree of spontaneous regression with the development of pigment spots. Others may show repigmentation of some lesions, extension of others and appearance of new lesions at other sites simultaneously. At times, the pigment spots that occur spontaneously or in response to therapy disappear, but may reappear. Complete spontaneous cure is

extremely rare. Sometimes residual depigmentation may be left behind after repigmentation of a large macule[20].

2.1.7 HISTOPATHOLOGY OF VITILIGO

Routine histopathology shows the absence of melanin granules in vitiliginous areas. Histochemical studies show a gross lack of dopa-positive melanocytes in the basal layer along with unusually large, highly dendritic, melanized melanocytes in the epidermis and numerous melanophages in the dermis of the border area. A dermal lymphocytic infiltrate has been reported at the active margins of a vitiligo macule along with extracellular granular deposits. The dopa reaction is not positive in amelanotic skin. Signs of degeneration of melanocytes and prominence of dendritic Langerhans cells and indeterminate cells have been demonstrated in electron microscopic studies.

Inactive melanocytes were found to have converted into active melanocytes in repigmenting areas. Decreased dendricity and increased intracellular space between melanocytes and keratinocytes have been reported in vitiliginous patches[20].

2.1.8 VITILIGO THERAPY

Modern treatments can be divided into the following categories:

I. General aspects

II. Medical treatment

III. Surgical treatment

IV. Cosmetic camouflaging

V. Bleaching

I. General aspects:

Patients should be told about the need for a good general health and a balanced nutritious diet rich in proteins, vitamin B-complex, vitamin E and minerals such as copper, zinc and iron.

They need to be instructed regarding avoidance of physical, chemical and emotional trauma as far as possible. Avoidance of soaps and detergents containing phenolic compounds, rubber goods and contact exposure to other chemical agents known to be detrimental to melanisation is advisable.

II. Medical treatment:

1. Psoralen compounds and Ultraviolet A (PUVA) therapy:

Topically, treatment using a combination of psoralen derivative or other photosensitizing agent, followed by irradiation with long wave ultraviolet light constitutes photochemotherapy. When it uses the psoralen group of drugs with

long wave UV irradiation, then it is called PUVA therapy. It is called PUVASOL therapy when sun exposure is utilized as the source of UVA.

2. UVB phototherapy:

Broadband UVB lamp increases the chance of phototoxicity and skin cancer. Narrow band UVB therapy obviates this and more effective than PUVA therapy. Other advantages are: a) since no oral medication is required, no side effects of psoralens are seen; b) can be used in pregnancy and childhood; c) no post-exposure eye protection is necessary and d) exposure time is shorter than with PUVA.

3. Excimer LASER:

The 308 nm xenon chloride excimer laser is an effective and safe modality for the treatment of chronic stable vitiligo. Good results are achieved in a short duration of time.

Treatment is given twice or thrice weekly for 10 – 15 sittings. Initial pigmentation is observed by 4-8 weeks. It acts through immunomodulation by affecting the T cells. Repigmentation is fastest with thrice weekly treatment.

4. Topical corticosteroids:

Topical corticosteroids have become the first line of treatment in patients with a few localized lesions.

Potent fluorinated corticosteroids yield results when used for a period of 2 to 4 months but side effects (atrophy, telangiectasia, striae) may develop, in which case, treatment needs to be interrupted. Percutaneous absorption may result in chronic adrenal insufficiency.

5. Oral corticosteroid therapy:

Systemic corticosteroid therapy has been advocated in the pulse form in order to reduce the side effects. Oral minipulse (OMP) therapy has been considered as a safe one.

III. Surgical treatment:

In selected cases of vitiligo or other types of leukoderma, there is a scope for surgical treatment. They are surgical excision, dermabrasion, micropigmentation, punch grafting, minigrafting, thin Thiersch grafting, epidermal grafting and transplantation of invitro cultured epidermis bearing melanocytes.

IV. Cosmetic camouflaging:

Suitable cosmetic preparation may help many patients by masking the achromic macules. Many proprietary cosmetics are available worldwide for different skin complexions.

V. Bleaching (or) depigmentation:

In extensive vitiligo with scattered pigmented islands of skin and without any hope of recovery, removal of remaining pigment to give the skin a uniformly white appearance may be cosmetically desirable. Depigmentation may be achieved with the use of a 20% cream of monobenzyl ether of hydroquinone for 3 – 6 months or longer.

2.2.1 HISTORY OF CLINICAL NEUROPHYSIOLOGY

The developments in clinical neurophysiology are closely linked to the discovery of electricity. Lugi Galvani was the professor of Anatomy at the University of Bologna. Galvani (1791) discovered that the nerves were the good conductor of electricity. His observations revealed the relationship between electrical stimulation of the nerves and muscle contraction. Galvani believed that electricity was generated by the body and channelled through the nerves.

Carlo Matteucci, Professor of Physics in Pisa, Italy, investigated the localisation of electricity in the muscle nerve preparation and proposed the concept of electrophysiology based functioning of the nervous system.

Duchenne, in 1833 was the first to systematically study the neuromuscular disease. He developed the equipments and techniques for electrical stimulation.

In 1850, Helmholtz succeeded in measuring the conduction velocity of nerve in frog by mechanically recording the muscle twitch.

In 1851, Du Bois Raymond recorded the action potential of voluntarily contracting muscle using jars of liquid as electrode. This was the beginning of eletctromyography.

The method of electro diagnosis, based on faradic and galvanic current was introduced by Erb in 1861. Erb was the first to demonstrate increased electrical irritability of motor nerves in tetany, which is known as Erb's phenomenon [4].

Within 25 years of publication of Raymond's book, Richard Caton who was a lecturer at Liverpool, UK, conceived the idea that the nerve impulse flows in and out of brain and its passage might be detectable. Caton has already published his results in 1875, on peripheral nerves and muscle currents and was in search of their cerebral counter parts. He noted that when both the electrodes were placed on the cortical surface, there is continuous waxing and waning of potentials which was unrelated to respiratory and cardiac rhythm. He also proved their biological nature by demonstrating the effect of anoxia and anaesthesia. These potentials were abolished by the death of the animal. As a result of these experiments, Caton had not only discovered electroencephalogram but also the cerebral potential changes evoked by sensory stimulation.

Adolf Beck at Department of Physiology, University of Jagiellonski, Poland, unaware of Caton's work 15 years earlier, was also investigating the electrical signals of brain in response to sensory stimuli. It was Neminsky, who gave the first photograph of evoked potential recorded from cortex of a cat following stimulation of sciatic nerve. Evoked potentials have emerged as an important electro diagnostic technique.

Richard Caton is credited for the first description regarding spontaneous and evoked potentials in experimental animals.

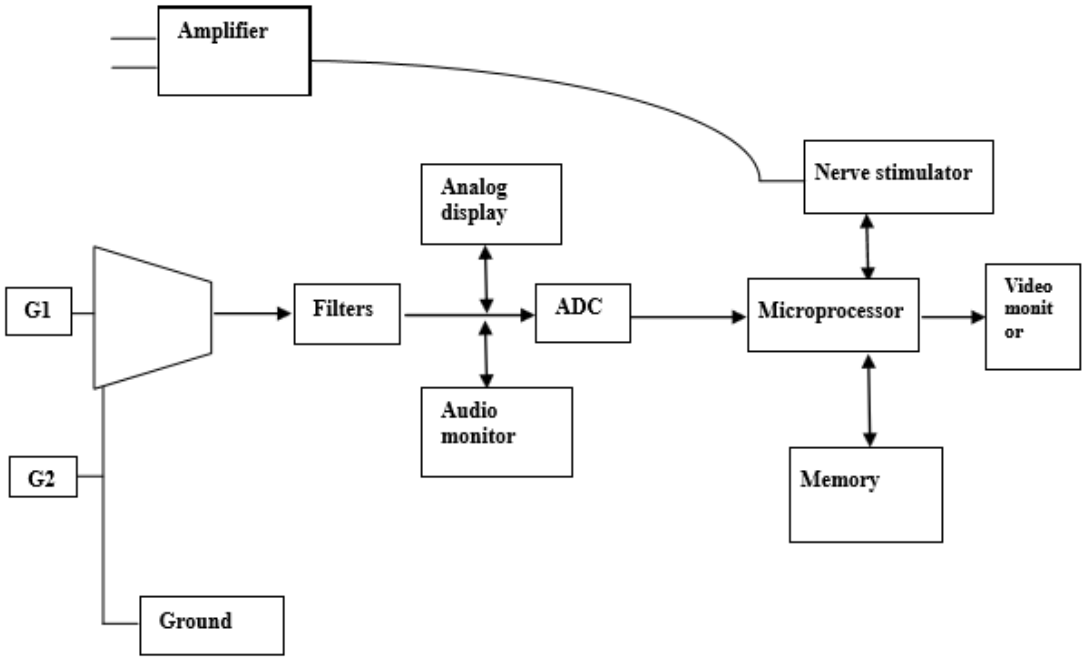
PA Davis had recorded auditory evoked potentials in 1939, but it was George Dawson, whose contributions such as photographic superimposition and averaging have helped the recognition of very small but time locked evoked responses.

The first human auditory brainstem evoked potential was fully described by Jewett and Williston in 1971. Sohmer and Feinmesser (1967) had recorded these waves earlier and attributed their origin to brainstem structures.

2.2.2 AN INTRODUCTION TO ELECTRO DIAGNOSTIC SIGNALS

The clinical electro diagnosis involves the recording, display, measurement and interpretation of action potentials arising from central nervous system.e.g.Evoked potentials [4]. The study is carried out using the commercially available equipments, which have a simple and user-friendly programme.

The major components of equipment are shown in Fig.1



Schematic diagram showing major components of electro diagnostic equipment. ADC – Analog-to-digital converter.

Biophysics:

Normal nerve cell membrane has a resting trans-membrane potential, because there is an uniform distribution of positively charged ions outside and negatively charged ions inside the cell membrane.

The potential difference between two points, either inside or outside the nerve cell membrane is zero in spite of a potential difference (voltage) across the nerve cell membrane. During depolarisation, voltage difference between two points occurs and is responsible for current flow along the cell membrane.

The action potential current is due to movement of sodium and potassium ions. The current is resisted by intervening tissue and this resistance is known as impedance.

Voltage = current x impedance

Voltage is not an absolute value, but represents the difference of voltage between two points, which can be positive or negative. In clinical neurophysiology, the action potential amplitude is expressed in milli volts (mV) or micro volts; current in milli-amperes (mA) and impedance in mega-ohms. Time measurements are in milliseconds (ms) or microseconds. The action potentials originating in nerves are displayed and interpreted as wave forms, in which amplitude varies with time.

To prevent distortion of signals, the recording system including the electrodes must respond to all the frequency components within the potential of interest and exclude the unwanted frequencies.

Electrodes:

In electro diagnostic tests, three electrodes namely active, reference and ground are used. The action potential is measured between active and reference electrodes and the ground electrode serves as zero voltage reference point.

The electrodes are made up of variety of metals and alloys such as stainless steel, platinum, silver chloride, nickel, chromium, silver and gold. An electrochemical reaction occurs, when a metal electrode interacts with an electrolyte such as sweat, electrode paste or fluid.

This results in an electrode polarisation potential of 100 – 600 mV. Silver – silver chloride electrode has the advantage of stable electrode polarisation potentials, which results in noise – free recording.

Both surface and needle electrodes are commonly used in electro diagnostic studies. The surface electrodes are in the form of disc, cup or ring. Gold -plated copper disc electrodes are used. The disc electrodes are used in the present study as they give better stability; most importantly they prevent the spread of infections. The surface electrodes are placed with the help of electrode paste which also provides an inter phase between the patient and equipment. The skin is cleaned and prepared by gently abrading with cleansing

jelly. The electrode paste is gently rubbed on the skin and then applied for optimal contact. The action potentials are recorded between active and reference electrodes.

Filter:

Filter is a device, which selectively resists the frequency domain of a signal. The filter band pass is the frequency range of a signal, which is transmitted through the filter. The frequency range in which a signal is rejected is known as stop band. Between the pass band and stop band is the transition band, which is characteristic of the filter.

Filtering of neuro physiological signals is required for eliminating the noise, optimizing the recording. The noise frequencies can be eliminated, if these are different from the frequency components of action potential. Filtering is also useful in bringing out the characteristics of waveforms, which may otherwise be not obvious.

The low frequency filters remove the slowly changing low frequency components and allow higher frequencies to pass through. Therefore, the low frequency filter is also known as high pass filter. Similarly high frequency filers eliminate the rapidly changing high frequency components and allow the low frequency to pass through. Hence the high frequency filters are called low pass filters.

The other method of filtering is digital filtering using mathematical algorithms applied to the signal after conversion into a digital form. The filters do lead to an alteration of waveforms because of their frequency attenuation and phase shift characteristics. Most signals of clinical interest contain significant frequency components near 50 – 60 Hz, but the use of notch filters to exclude noise (50 – 60 Hz) is not recommended because of distortion of the action potential.

The recommended filter settings and low cut and high cut filters in auditory brainstem evoked potential are low cut 10 – 100 Hz and high cut 3 kHz [45].

Amplifier:

Since the biological signals are very small, a variable degree of amplification (up to 500,000 times) is needed before being displayed. The electrode impedance includes intrinsic impedance of the electrode and the impedance of electrode–skin interface. For recording action potential, the action potential generated in central nervous system or nerve must flow through the electrode into the amplifier and return to the subject through the ground lead. Electrode impedance results in drop of the amplitude of action potential. This attenuated action potential reaches the amplifiers.

To reduce this attenuation, the impedance of the amplifiers must be much greater than the electrode impedance. To limit the drop in signal

amplitude by 1%, the amplifier impedance should be 100 times the electrode impedance.

The needle electrodes have higher impedance than surface electrodes. The electrode and amplifier impedance both are inversely related to the frequency. A 100:1 ratio of electrode to amplifier impedance should be maintained across the range of frequencies contained in the waveform under study. This minimises the waveform distortion and improves noise rejection. Unequal electrode impedance imbalances the electrode amplifier input, converting some of the noise into different signal, which is amplified to the same extent as the neuro physiological signal. To reduce the impedance-induced noise, the active, reference and ground electrode impedance should be minimized and balanced.

Averager:

Averaging is a mechanism to extract very small signals, which are buried in larger noise, as evoked potentials are buried in EEG. By averaging, the signals which are time-locked become prominent and the noise which is randomly occurring is cancelled out. The time locked sequential responses are digitized and stored in the memory of the equipment. The sequential responses are mathematically summated, averaged and displayed. The signal-to-noise ratio (SNR) depends upon the number of responses averaged.

$$\text{SNR} = \frac{\text{Signal amplitude x Number of sweeps}}{\text{Noise amplitude}}$$

Using a sweep rejection criterion, the time locked noise signals can be rejected. The sweep is rejected if the incoming signal exceeds predetermined amplitude, which is expressed as a percentage of the input range of the amplifier. Typically, sweep is rejected when the signal exceeds 95% of the input range. It is important to appreciate the relationship between SNR and the number of sweeps averaged. The number of sweeps have to increase four times to double the SNR.

Display:

For digital display, an analog-to-digital converter (ADC) and digital processing techniques are required. This facility is available in all modern electro diagnostic equipments. The continuously varying neurophysiological signal is sampled at discrete time intervals and the amplitude of the signal is converted into a number, following amplification and filtering. This process is known as analog to digital conversion. The unit of an ADC is a bit, which is a binary digit. The resolution capacity of an ADC is related to the number of bits.

$$\text{Resolution of ADC} = 2^{n} \text{ (n = number of bits)}$$

An ADC with 6 bits will resolve 64 levels, with 12 bits will resolve 4096 levels and hence, the later will have better resolution. The polyrite

Medicaid Neuro Perfect Plus has 14 bits, that has still higher resolutions of 16484 levels.

In digitalised display, certain details of the waveform may be lost because of the limited number of pixels used for display.

The advantage of this technique is that the signals can be redisplayed with greater sensitivity without loss of waveform accuracy. The series of digital numeric values derived from ADC, must accurately reflect the amplitude, morphology and variability in the original waveform. This depends on sampling rate of ADC, which should be fast enough to capture the rapidly changing features of the waveform. The minimum sampling rate should exceed twice the value of high frequency components of the waveform. This criterion is Nyquist criterion, which sets the lower limit of analog to digital conversion rate. Insufficient sampling rate results in distortion of waveform (Aliasing effect). The sampling done as per Nyquist criterion, may still not ensure full representation of waveform, therefore more rapid sampling rate (5 – 10 times the highest frequency of the signal) is required.

Sweep time:

On high gain or sensitivity, the latency measurements are shorter, because it becomes possible to visualize smaller take offs from baseline. The changes in peak latency are less affected by sensitivity, because of easy identification of the peak compared to the onset of action potential. Similarly, duration of action potential increases as the display sensitivity is increased.

Increase in sweep speed results in shortening of latency, although this effect is variable and is of small magnitude. In digital display, the horizontal display resolution is limited by the number of horizontal pixels in each given division. It is possible to have automatic cursor placements for the measurement of latency, duration and amplitudes. Such measurements should be visually monitored so that noise and other artefacts are not included in the measurement.

Stimulator:

The stimulators are required for evoked potential studies. For reducing the imbalance of impedance between active and reference electrodes, electrically isolated stimulators, matched recording electrodes with good skin preparation, pacing the ground electrode between stimulating and recording electrodes, avoiding a bridge between electrodes by electrode jelly or sweat and use of short cables for stimulating and recording are helpful.

Evoked potential:

Electrical potentials that occur in the group of neurons in response to the stimulation of a sense organ, which can be recorded by surface electrodes are known as Evoked Potential.

Brainstem Auditory Evoked Potential:

Brainstem auditory evoked potentials typically use a click stimulus that generates a sequential activation of brainstem auditory pathway i.e.,

response from the hair cells of cochlea, the signal travels along the auditory pathway from the cochlear nuclear complex to the inferior colliculus in midbrain generating wave I to wave V. They occur 10 msec after auditory stimuli.

Brainstem Auditory Evoked Potentials / Auditory Brainstem Response (ABR) / Brainstem Evoked Response Audiometry / Far-Field Electrocochleography:

The term brainstem auditory evoked potentials are not completely accurate because the roster of generators includes distal (with respect to the brainstem) cochlear nerve and also the thalamocortical auditory radiations, neither of which is within the brainstem. Other synonyms include auditory evoked brainstem response, far-field electro-cochleography and brainstem evoked response audiometry[45]. The procedure of evoked response audiometry is same as that of ABR. In audiometry, the evoked response is obtained with different intensities of click stimuli. Physiologic testing of the function of the eighth cranial nerve and its connections provide sensitive, quantitative measures of mild or early damage to the peripheral or central components of the nervous system[46].

2.2.3 Role of electrophysiological studies in vitiligo

Nikifordis et al. 1993, studied the auditory brainstem response in 30 patients with vitiligo to detect the subclinical auditory abnormalities.

Their findings revealed a statistically significant ($p < 0.01$) reduction in first peak latency and a statistically significant ($p < 0.01$) increase in I – III inter peak latency in the patients when compared with that of controls [10].

The decrease in first latency may be due to the decrease in number of active melanocytes in the inner ear and the resulting impairment of ionic exchange between perilymph and endolymph. The prolonged I – III inter peak latency may be due abnormal synaptic activity and transmission of action potential from the auditory nerve to the superior olive.

Bassiouny et al. 1998, performed BERA in vitiligo subjects. He found a statistically significant decrease in latency of wave I, which may be due to mild cochlear lesion in the melanin lacking individuals. The observed increase in I - III inter peak latency was due to decrease in wave I latency, as the absolute latency of wave III in their study was within normal limits [48].

Ozuer et. al 1998, performed BERA in 50 individuals with vitiligo and compared the results with that of 50 heathy controls.

With regard to latencies and amplitudes, there was no statistically significant difference between both the groups. They suggested post-mortem histopathological study of the inner ear and brainstem in vitiligo subjects to understand the pathology [12].

Aydogan et al. 2005, studies brainstem auditory evoked response of 57 vitiligo patients and healthy controls. The I, III and V latencies and inter peak latencies of I – III, III – V and I – V were compared between the groups.

A statistically significant increase in the latency of wave III and inter peak latency I – III in both the ears and a significant increase in the latency of wave V in the right ear were noted.

The prolonged third and fifth peak latency and I – III inter peak latency were related to their superior olivary nucleus (SON) and upper brainstem or inferior colliculus pathology.

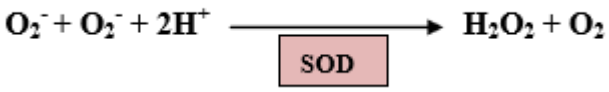
This may be due to synaptic activity abnormality and abnormal transmission of action potential from the auditory nerve to the cochlear nucleus and from the cochlear nucleus to the SON and inferior colliculus. Hence, vitiligo may be associated with delay in synchronization of action potentials in these nuclei [49].

Shalaby et al. 2006, studied the auditory function affected in vitiligo patients with different distribution and duration. They explained

that the disorders of hypopigmentation for a long duration may cause the degeneration of outer hair cells degeneration both structurally and functionally beginning from the basal turn of cochlea with intact inner hair cells. Another explanation was about endolymph calcium levels. The calcium levels in endolymph increased from the base to the apex and endocochlear potentials decreased towards apex. There was no significant calcium gradient noted in albinos and there was less endocochlear potential decline. These results confirm the involvement of melanin in the calcium active transport into the endolymph. In their study, there was no statistically significant difference ($p > 0.05$) between cases and controls. They concluded that some of the vitiligo patients may have auditory defect depending on individual's susceptibility, residual melanocyte number in the inner ear and nature of immunological abnormalities[50].

2.3.1 SUPEROXIDE DISMUTASES IN OXIDATIVE STRESS

Superoxide dismutases (SODs) are metalloenzymes that catalyze the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide and form a crucial part of the cellular antioxidant mechanism[66].



SODs are classified into three types according to their metal content : copper / zinc (Cu / Zn), iron (Fe) and manganese (Mn). SOD is distributed

widely in both plants and animals. It is present in higher concentrations in liver, heart, kidney and erythrocytes. There are three forms of SODs in human beings. They are mitochondrial MnSOD, cytosolic Cu/Zn-SOD and extracellular SOD[67]. Extracellular SOD is found in the interstitial spaces of tissues and in the extracellular fluids, which accounts for the majority of the SOD activity in the lymph, synovial fluid and plasma [68,69].

The amount of SOD in cellular and extracellular environments is important for the prevention of diseases linked to oxidative stress. The reaction catalysed by SOD is very fast, having a turnover of $2 \times 10^9 M^{-1}sec^{-1}$ and the sufficient amount of enzymes present in the cells and tissues, keep the superoxide concentration(O_2^-) very low [66].

Catalase is a hemoprotein. It contains four heme groups. Catalase is found in blood, bone marrow, mucous membranes, kidney and liver. Inside the cell, it is found in peroxisomes. It catalyses the conversion of hydrogen peroxide into water and molecular oxygen (Harper, 2000).

$$2H_2O_2 \xrightarrow[\text{Catalase}]{} 2H_2O + O_2$$

2.3.2 Oxidative stress in vitiligo

There are several studies on the oxidative stress in vitiligo. Oxidative stress may be due to excess production or inadequate removal

of reactive oxygen species (ROS) or free radicals. A radical is a molecule having an unpaired electron [15,51]. Superoxide, nitric oxide, hydroxyl, alkoxyl and alkyl - peroxyl (lipid) are radicals. Reaction of a radical with a non radical, forms new radicals, which in turn react with further macromolecules [15].

In a healthy body, the oxidant molecules are removed by antioxidant systems. Cellular antioxidant defences are dismutase, peroxidise and catalase enzymes[15]. Superoxide dismutase (SOD) which is present in cytoplasm and mitochondria catalyses the dismutation of superoxide to hydrogen peroxide and oxygen[15,51]. Beta carotene, ascorbic acid, tocopherol, uric acid, coenzyme Q and ferritin are major antioxidants. Beta carotene, coenzyme Q and vitamin E are low molecular weight antioxidants and they are present in the cell membrane. Their deficiency causes accumulation of ROS leading to lipid peroxidation, DNA mutation, activation or inactivation of enzymes and oxidation of protein [52].

The inherent capacity of cells to withstand the oxidative stress is due to several factors: Their ability to repair oxidatively modified biomolecules, their antioxidant ability and their capacity to sustain metabolic requirements by deriving energy from alternate pathways.

Recently, several studies on superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and nitric oxide (NO) have been

done and different results were reported about these biomarker levels. Some researchers observed high levels of oxidants and antioxidants. Some others reported no difference between patients and controls. Some studies have reported even low levels of these markers.

Yildirim et al. 2004, Damma et al. 2009 and Sravani et al. 2009 found high levels of superoxide dismutase and glutathione peroxidase levels and low levels of catalase [53,54,55] in the vitiligo patients when compared with that of controls.

Koca et al. 2004, and Akrem et al. 2009, found low levels of superoxide dismutase, catalase and glutathione peroxidise in the vitiligo patients when compared with that of controls [56,57].

Picardo et al. 1994, and Passi et al. 1998, found no difference in blood levels of superoxide dismutase, glutathione peroxidise, lipoperoxidase, vitamin E and ubiquinone between vitiligo subjects and controls [58,59].

Eventhough these studies had different results, all of them suggest an imbalance between anti-oxidant and oxidant systems in vitiligo. At this point, further studies on a larger population are necessary to prove the role of these markers in the etiopathogenesis.

3. AIM AND OBJECTIVES

Aim of the study was

1. To evaluate the conduction in the auditory pathway in vitiligo subjects.

2. To study the role of oxidative stress in vitiligo subjects.

Objectives of the study were

1. To evaluate the audiological abnormalities using Brainstem Evoked Response Audiometry as a tool in individuals with vitiligo.

2. To study the role of oxidative stress by assay of serum superoxide dismutase enzyme level in individuals with vitiligo.

3. To do a comparative study in a healthy control group.

4. MATERIALS AND METHODS

The study was conducted in the DEPARTMENT OF PHYSIOLOGY, STANLEY MEDICAL COLLEGE, CHENNAI – 1, from 2014 to 2015 after getting approval from the Institutional Ethical Committee, Stanley Medical College, Chennai – 1.

4.1 SELECTION OF SUBJECTS

4.1.1 Study group:

40 individuals affected by vitiligo of various dermatomes and distribution were recruited from the Department of Dermatology and Department of Cosmetology, Stanley Medical College , Chennai-1.

Inclusion criteria:

1. Clinically diagnosed vitiligo patients.

2. 15 - 40 years of age group.

3. Both gender.

Exclusion criteria:

1. Age less than 15 years and more than 40 years.

2. Subjects with any other chronic dermatological diseases including depigmentation disorders.

3. Subjects who were exposed to risk factors known to be responsible for sensory neural hearing loss eg. ototoxic drug intake, chronic noise exposure, head trauma etc.

4. External or middle ear diseases.

5. H/O ear surgery.

6. Any history suggestive of anaemia, diabetes mellitus, hypertension, thyroid disease and other autoimmune diseases.

4.1.2 Control group:

40 age and gender matched healthy controls were selected from the volunteers among the hospital staffs and accompanies of patients.

Study Design: Case control study

Place of study:

Neurophysiology Laboratory of Research Wing, Department of Physiology, Stanley Medical College, Chennai – 1.

4.2 METHODOLOGY

4.2.1 BRAINSTEM EVOKED RESPONSE AUDIOMETRY:

Brainstem Auditory Evoked Potential **(**BAEP) recording was done by using the instrument, Neuro Perfect Plus Medicaid Polyrite Hardware, Solokraft, India.

After taking a brief history, clinical examination was done. All the study subjects were examined under good light. Detailed dermatological examination including size, shape, number, colour and distribution of lesions was done. The individuals were made to relax and be comfortable prior to the test. The complete procedure and objectives of the study were explained in detail to the individuals in their regional language. The informed and written consent (Annexure I) was obtained from the individuals. The complete examination of both the external ears was done and wax was removed. Then pure tone audiometry was done in the ENT department, Stanley Medical College Hospital, Chennai-1.

Both the vitiligo subjects and controls had a prestructured proforma completed (Annexure II). Venous blood sample was collected from both the vitiligo and control groups for the estimation of superoxide dismutase enzyme.

Brainstem auditory evoked potentials were recorded in 40 healthy individuals of both gender with age ranging from 15 to 40 years. In order to establish the reliability of the method, several repetitions of Brainstem Auditory Evoked Potential recordings were performed on different days and at different hours. BAEPs were recorded in 40 individuals of both gender affected by vitiligo of various dermatomes and distribution, in the age group ranging from 15 – 40 years.

4.2.1.1 PRE–REQUISITES:

- The subjects were clearly instructed to have shampoo head bath.
- The subjects were instructed to avoid oil on hair or hair spray.
- The subjects were instructed to avoid beverages and strenuous exercise on the day of recording.
- The subjects were made to get accustomed to research laboratory and be relaxed.
- The research room was made comfortable and noise free.
- Uniform normal room temperature was maintained.
- The subjects were grounded properly.

4.2.1.2 INSTRUMENT SETTINGS FOR RECORDING BAEP

1. Recording Electrodes:

The gold plated copper disc electrodes for recording BAEPs were used. No significant difference in latency and amplitude have been observed when BAEPs were recorded either with needle or surface electrodes. Surface electrode recordings were preferred because they were painless, had better stability and less chances of infection. 1 cm disc electrodes filled with conducting paste were used. The electrode impedance was kept below 5 kilo ohms. The BAEP was recorded from the ipsilateral ear with reference to the vertex. The two channels which were recorded from the ipsilateral and contralateral mastoids are referred to as A_i and A_c respectively. Reference electrode is $C_{z..}$ Ground electrode is $F_{z.}$ Vertex is the suitable location since waves II – V have good amplitude with little muscle artefact.

2. Montage Chosen:

- Active electrode – Ipsilateral mastoid: A_i
- Reference electrode – Vertex: C_z
- Ground electrode - a point in front of the reference electrode: F_z

3. Amplifier and Averager:

BAEPs were recorded using an amplification of 200,000 – 500,000. Two to three repetitions were done and superimposed to check for reproducibility. BAEP repetition was superimposed exactly. A 10 ms epoch after the click stimulus was averaged, amplified and displayed on the monitor.

4. Filter settings:

The low filter was set at 100 Hz and high filter at 3,000 Hz.

5. Auditory stimulation:

The BAEPs were produced by a brief click stimulus, which is a square wave pulse of 0.1 ms duration. The pulse moves the ear phone diaphragm away from the subject's ear which is a rarefaction phase stimulus. Wave I amplitude is greater with rarefaction compared to condensation stimulus. Since recognition of wave I is very important, rarefaction click polarity was chosen. Clicks were usually presented 10 – 70 times per second.

The slower or intermediate rates were preferred because waveforms are well defined. A click rate of 11Hz was used. The click stimulates not only the ipsilateral ear, but also travels via bone and air conduction to stimulate the contralateral ear at an intensity of 40 – 50 dB

lower than the ipsilateral ear. White noise at 30 – 40 dB blocks the stimulation of contralateral ear thus prevents false BAEP responses.

4.2.1.3 Procedure:

BAEP recording was done in Neurophysiology Laboratory of Research Wing, Department of Physiology, Stanley Medical College using Neuro Perfect Plus – Medicaid Polyrite Hardware, Solokraft, India. The left and right ears were tested separately in all the subjects. The laboratory temperature was maintained uniformly. The recordings were done in the forenoon between 10 am to 12 noon in sitting posture two hours after a light breakfast. The subject was asked to recline on a chair comfortably with their feet placed on a wooden board.

In order to avoid hearing defect interferences, pure tone audiometry was done at ENT department, Stanley Medical College Hospital. The body temperature was measured, since hypo or hyperthermia may cause alterations in latencies of both absolute and inter peak. The disc electrodes were placed on scalp by 10 – 20 standard system with conducting jelly. The skin and electrode impedance were checked. Since the potentials recorded are in far field, well displaced from the site of impulse generation, the waveforms recorded were very weak and hence amplified. The amplification is achieved by improving the signal : noise ratio. To improve signal to noise ratio, three parallel approaches are designed to achieve the goal.

Filtering:

This is employed to reduce the recording bandwidth, so that only the important components of the signal are recorded.

Repeated stimulation:

This is done with synchronous time domain averaging to increase the amplitude of the components of the signal. In real time situations, these two can be achieved by connecting the recording electrodes to a pre amplifier, with appropriate filter settings.

Polarity alteration:

By altering the polarity of impulses recorded, the artefacts are cancelled making the brainstem waves to stand out. The earthing electrode is important for proper functioning of pre amplifier.

The automatic artefact rejection was used. The sweep velocity was 1 ms. The click acoustic stimuli at a rate of 11 pulse per second at an intensity of 80 to 90 dB hearing level was given to the ear stimulated, was given through head phone supplied by Medicaid. The continuous 1000 auditory click responses were summated, averaged and displayed. All the techniques of measurement, duration and research laboratory temperature were maintained uniformly throughout the study [45].

4.2.1.4 PARAMETERS STUDIED:

The Absolute wave latency I, II, III, IV and V, Inter Peak Latency I – III, I – V, III – V and amplitudes of waves were measured. The wave pattern in BAEP recording is 5 or more peaks within 10 ms of stimulus. Initial five peaks have clinical significance. The succeeding peaks VI – VIII are quite variable and are not clinically useful. In normal subjects, waveform IV – V forms complex. Absolute amplitudes are too variable to be of any clinical use even in inter ear comparison.

Electro physiological study:

Brainstem auditory evoked potentials are the potentials recorded from the ear and vertex in response to a brief auditory stimulation.

It is used to assess the conduction through the auditory pathway up to the midbrain. Brainstem auditory evoked potentials comprise of 5 or more peaks within 10 ms of the stimulus[4].

The auditory nerve and brainstem auditory evoked potentials are volume conduction to surface electrodes. At the vertex and ear lobe, these form vertex positive and vertex negative waves which are known as brainstem auditory evoked potentials. The peak to peak amplitude of these waves recorded from the scalp is only about $1/100^{th}$ the amplitude of ongoing spontaneous EEG activity. There are 5 or more distinct waveforms recorded within 10 ms of the auditory stimulus. By

convention, BAEPs are recorded positive up, which is the opposite of most electro physiological recording.

Origin of brainstem auditory evoked potentials:

Waveform I – Vestibulo cochlear nerve

Waveform II – Cochlear nucleus

Waveform III – Superior olivary nucleus

Waveform IV – Lateral lemniscus

Waveform V – Inferior colliculi

4.2.1.5 NORMAL BAEP

Classical BAEP consists of 5 – 8 vertex positive peaks, which are labelled using Roman numerals. The troughs, immediately following peaks are designated by the same numerical with a prime mark. The important features in recognition of different waveforms are as follows:

Wave I:

Is a prominent initial up going peak in the ipsilateral ear-recording channel. It appers 1.4 ms after the stimulus and is markedly attenuated or absent from the contralateral ear-recording channel. Patients with only central nervous system problems, will have a normal wave I since it originates from VIII nerve. Conversely, the patients with

significant peripheral hearing impairment may have a poorly formed or absent wave I, but relatively normal wave II to V.

Wave II:

Is poorly defined in some adults. It appears as a small peak along the down going slope of wave I or in the up going slope of wave III. Sometimes the fusion of waves II and III results in M shaped II – III complex.

Wave III:

It is usually a prominent peak and is followed by a prominent III trough. In the contralateral channel, wave III often appears smaller and earlier than the ipsilateral ear because its amplitude is similar at the vertex and contralateral ear. This feature may help in wave III recognition. Some normal subjects have a bifid wave III, which is associated with a normal I-V IPL. In some adults, wave IV may normally be closer to wave III than to wave V and give an appearance of bifid wave III.

Wave IV and V:

Wave V is the most prominent peak appearing 5.5 ms after the stimulus. It starts above the baseline and its trough is maximal below the baseline. On ipsilateral recording, wave V fuses with IV, resulting in

a wave IV-V complex. The wave IV and V tend to be separated on contralateral recording.

The wave IV and V may have the following patterns:

- Single peak which is completely fused as a tall wide pyramid, whose base should be more than 1.5 ms.
- Two peaks which are close but still visibly separated.
- Wave IV may be on the up going slope of wave V.
- Wave V may be on the down going slope of wave IV.

Measurement and Normal Values of BAEPs:

The following parameters are measured for the analysis of BAEP:

- Absolute latency and amplitude
- Inter Peak Latencies (IPLs)
- Amplitude ratio of wave V/ I or IV – V complex / I
- Inter ear inter peak differences

Absolute Latencies and Amplitudes:

Absolute latency is measured as the distance (expressed in ms) from the beginning of the first wave to the peak of that wave. The difficulty is encountered when the waveforms are poorly formed. The method recommended for latency comparison is to superimpose the major ascending and descending limbs of waves of two sides against a strong

source of light. Absolute amplitude is measured as the height (expressed in μv) from the peak of the wave to the trough of that wave. Absolute amplitudes are too variable to be of any clinical use even in inter ear comparison.

Inter Peak Latency:

The commonest IPLs employed in clinical practice are I - V, I – III and III – V.

I – V IPL:

The latency difference is a measure of conduction from proximal VIII nerve through pons to midbrain. The typical upper limit of normal I-V IPL is 4.5 ms. This IPL is slightly shorter in young women and longer in older women. Normal right to left asymmetry should not exceed 0.5 ms. The I – V IPL can be prolonged in a variety of disorders including focal damage produced by demyelination, ischemia, tumours or diffuse diseases such as degenerative disorders, hypoxic brain damage, etc.

I – III IPL:

The latency difference between wave III and I is a measure of conduction from VIII nerve across the subarachnoid space into the core of lower pons. The I – III IPL can be increased in any diffuse process

that affects the generation of these waves. The I – III IPL is more susceptible to tumour, inflammation or other disorders affecting the proximal portion of VIII nerve, ponto medullary junction or the lower pons around superior olive or trapezoid bodies. Infarction can also prolong I – III IPL and this can also occur in meningitis and subarachnoid haemorrhage. The upper limit of normal for I – III IPL is about 2.5 ms and right to left symmetry is less than 0.5 ms. Excessive prolongation of I – III IPL should be associated with corresponding prolongation of I – V IPL.

III – V IPL:

It is a measure of conduction from lower pons to midbrain. It is influenced by contralateral conduction, although contribution from ipsilateral brainstem auditory pathway is also suggested. Its upper limit is up to 0.5 ms. Isolated prolongation of III – V IPL is not considered abnormal, unless associated with prolonged I – V or abnormal V/I amplitude ratio.

V/I amplitude ratio:

The V/I amplitude ratio may be influenced by filter setting and click intensity. Therefore, comparison with the normal values of the respective laboratory is essential [46].

4.2.1.6 ABNORMAL BAEP

BAEP abnormalities will include one or more of the following.

- Absence of waveforms
- Abnormal absolute or inter peak latencies
- Amplitude ratio abnormality
- Right to left asymmetry

Waves I – V:

Seen in most normal subjects, in which wave IV may form a wave IV– V complex. In such case, absence of wave IV does not indicate abnormality. Wave II can be difficult to distinguish in some normal subjects. By changing the stimulus intensity, rate and phase, wave II can be identified in normal subjects. Absence of all the waves (I – V) is abnormal. It is also considered abnormal, if the wave I only is recorded and the other succeeding waves are not recorded. Similarly, recordable wave I and III, but unrecordable wave IV and V is also abnormal. These conclusions are valid in a technically satisfactory recording, in which the background noise has been eliminated. The right to left latency asymmetry exceeding 0.5 ms is also abnormal.

Clinical neurophysiological correlation:

BAEPs have been correlated with different areas in the brainstem auditory pathways. Although the changes in BAEPs are non- specific, they provide information about the function of corresponding auditory pathway. The abnormalities of BAEP should therefore be correlated with the clinical picture and other investigations.

Although the BAEP has been studied in numerous neurological disorders, the most important clinical applications are in cerebellopontine angle tumour, intrinsic brainstem tumour, multiple sclerosis, coma and brain death [47].

4.2.2 ESTIMATION OF SERUM LEVELS OF SUPEROXIDE DISMUTASE

Collection of blood samples

About 5ml of venous blood was collected in a plain test tube from each individual participated in the study. Blood was allowed to clot for 30 min. at 25° C. The blood was centrifuged at 2000 x g for 15 min. at 4° C. The top yellow serum layer was pipetted out without disturbing the white buffy layer. The serum was stored in deep freezer at - 80 degree Celsius, till the estimation of superoxide dismutase levels.

Estimation

Estimation was done using Superoxide Dismutase kit, Lot Number: 0471696, Cayman Chemical Company, 1180 E. Ellsworth Rd. Ann Arbor, MI 48108. (Refer photograph 2).

Contents of the kit

1. Assay Buffer - Hypoxanthine and DTPA (Diethylene triamine penta acetic acid. Used to dilute the radical detector.

2. Radical Detector – Tetrazolium salt solution.

3.Sample Buffer – Used to prepare the SOD standards and dilute the xanthine oxidase and SOD samples prior to assaying.

4. Xanthine Oxidase

4. SOD Standard

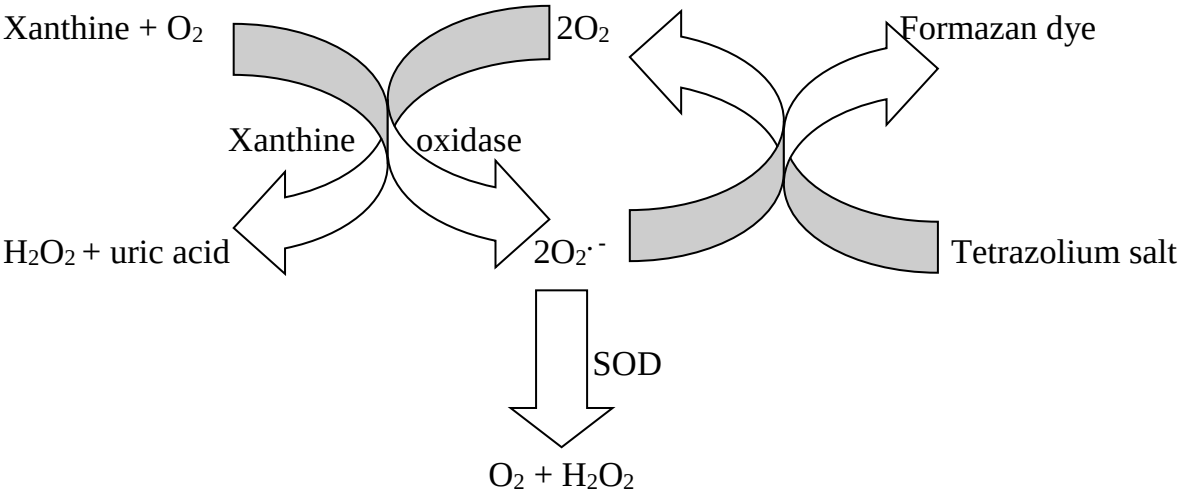
5. Sample Diluent

Principle

The function of Superoxide dismutase (SOD) is to catalyse the dismutation of the toxic superoxide radical to hydrogen peroxide and molecular oxygen. This method utilizes a tetrazolium salt for detection of superoxide radicals generated by hypoxanthine and xanthine oxidase which react with tetrazolium to form a red formazan dye. One unit of

SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. The SOD assay measures all three types of SOD (Cu/Zn, Mn and FeSOD). This method provides a simple, reproducible and fast tool for assaying SOD activity in serum.

Scheme of the Superoxide dismutase Assay :



5. RESULTS

In this study, Brainstem Auditory Evoked Potential was recorded in 40 vitiligo individuals and compared with 40 age and gender matched healthy normal subjects. BAEPs of both the ears were recorded. Absolute and Inter Peak Latencies were measured.

Statistical analysis : The Data were expressed as mean $\pm$ standard deviation. The variations in parameters between the two groups were tested using student independent t-test using SPSS software version 17. Statistical significance was tested using P value.

P - Value $<$ 0.05 - Significant

P - Value $<$ 0.01 - Highly significant

The mean age was 28.7 $\pm$ 6.92 years, with a range of 15 to 40 years in vitiligo subjects. The mean age was 28.45 $\pm$ 7.32 years, with a range of 15 to 40 years in the control group.

Age wise distribution of the study and control groups is depicted in Table 2 and Graph 1. Among the vitiligo subjects, 20% of them were in the age group of 16 - 20 years, 40% were in the age group of 21 - 30 years and the remaining 40% were in the age group of 31 - 40 years.

The mean duration of vitiligo was found to be 3.57 $\pm$ 2.11 years, with a range of 1 to 10 years. In this study, 24 (60%) were female out of 40 vitiligo subjects as depicted in Table 3 and Graph 2.

Distribution of various types of vitiligo is depicted in Table 4 and Graph 3. In our study, 45% of vitiligo subjects had vulgaris type ; 25% of them had acrofacial type ; 20% had segmental type of distribution and 10% had focal type of vitiligo.

The mean level of absolute wave latency III of left ear of vitiligo individuals was significantly prolonged when compared to that of the normal healthy controls as depicted in Table 5 and Graph 4.

The mean level of absolute wave latency III of right ear of vitiligo individuals was prolonged and found to be highly significant when compared to that of the normal healthy controls as depicted in Table 7 and Graph 6.

The mean level of Inter peak latency I - III of left ear of vitiligo individuals was prolonged and found to be highly significant when compared to that of the normal healthy controls as depicted in Table 6 and Graph 5.

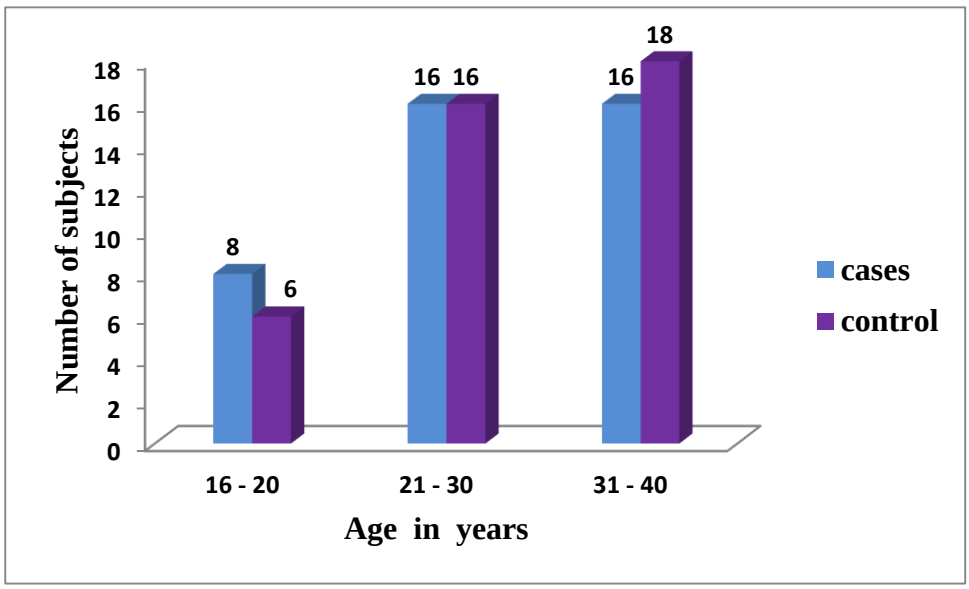
The mean level of Inter peak latency I - III of right ear of vitiligo individuals was prolonged and found to be highly significant when

compared to that of the normal healthy controls as depicted in Table 8 and Graph 7.

In this study, there was a decrease in the mean level of serum superoxide dismutase enzyme in the vitiligo subjects, when compared with that of the normal healthy controls as depicted in Table 9 and Graph 8. However, this was not statistically significant.

Table 2 : Age wise distribution of vitiligo individuals and controls

Age in years	Vitiligo individuals – n	%	Controls – n	%
16 - 20	8	20	6	15
21 - 30	16	40	16	40
31 - 40	16	40	18	45
Total	40	100	40	100

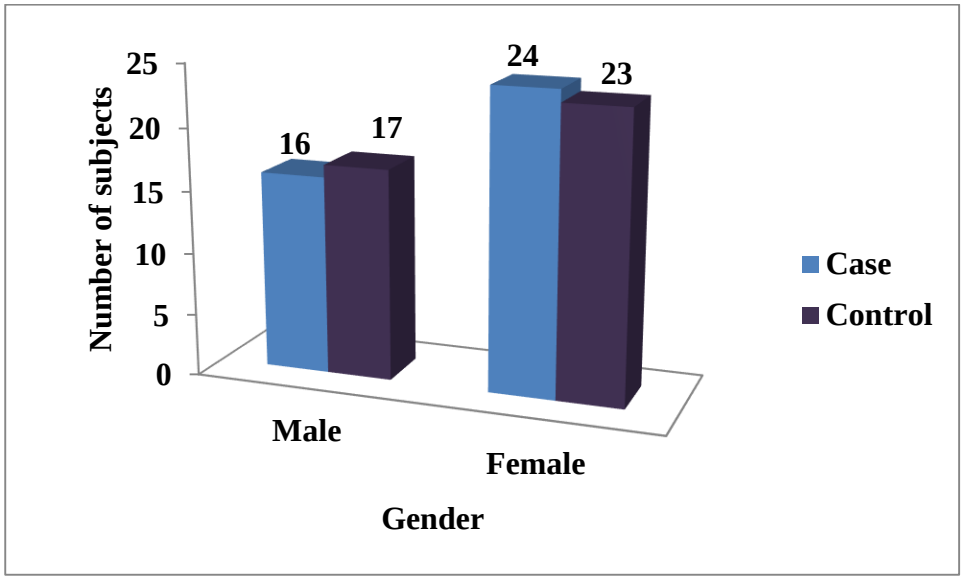


Graph 1

Age wise distribution of vitiligo individuals and controls

Table 3 : Gender distribution in vitiligo individuals and control group

Gender	Vitiligo individuals n	%	Control group N	%
Male	16	40	17	42.5
Female	24	60	23	57.5
Total	40	100	40	100

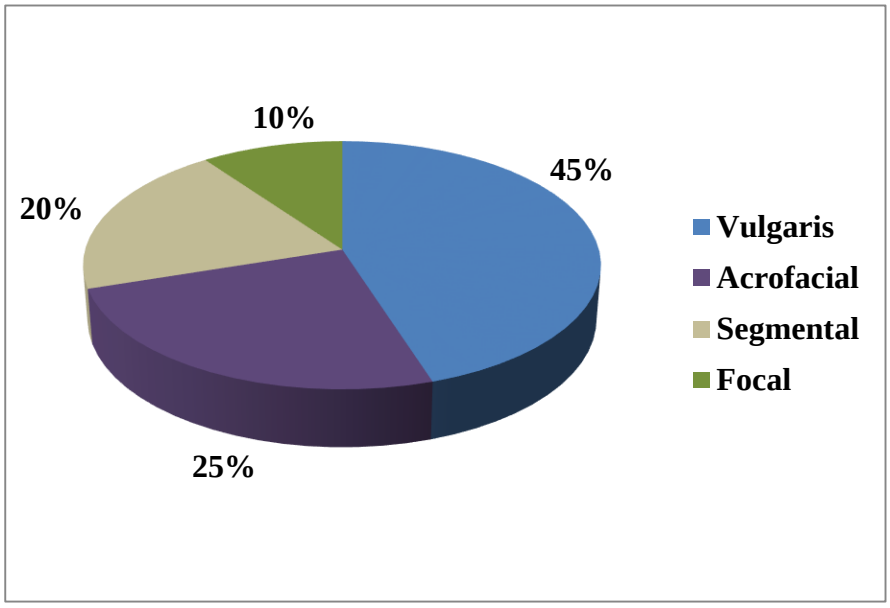


Graph 2

Gender distribution in vitiligo individuals and control group

Table 4 : Distribution of various types of vitiligo

Vitiligo types	Vitiligo subjects n = 40	%
Vulgaris	18	45
Acrofacial	10	25
Segmental	8	20
Focal	4	10



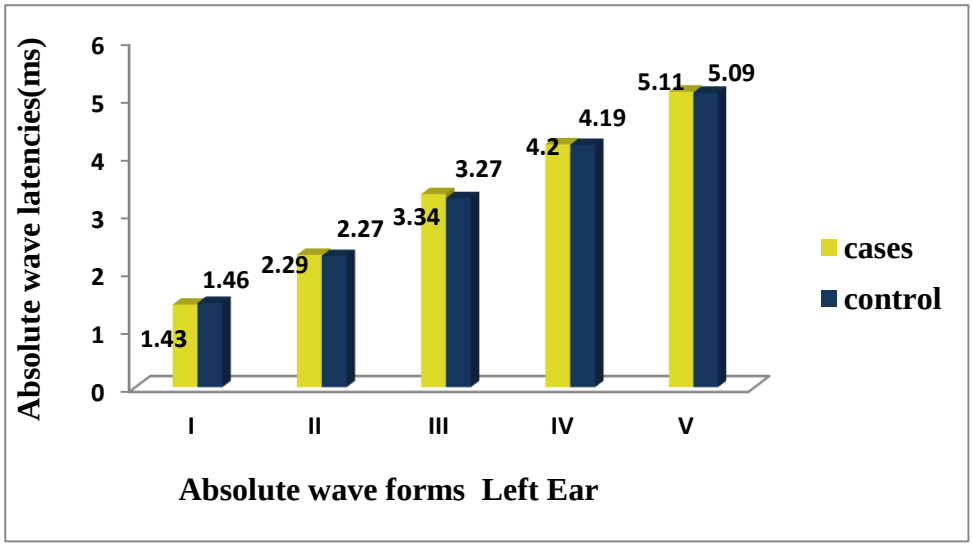
Graph 3

Distribution of various types of vitiligo

Table 5 : Absolute wave latencies of left ear compared between vitiligo individuals and control group

Absolute wave forms	Absolute wave latencies (ms)		P value
	Vitiligo individuals n=40 Mean ± SD	Controls n = 40 Mean ± SD	
I	1.43 ± 0.08	1.46 ± 0.07	0.37
II	2.29 ± 0.18	2.27 ± 0.15	0.57
III	3.34 ± 0.16	3.27 ± 0.19	0.01*
IV	4.20 ± 0.19	4.19 ± 0.17	0.67
V	5.11 ± 0.13	5.09 ± 0.13	0.38

*P < 0.05 - significant ; Student independent t – test

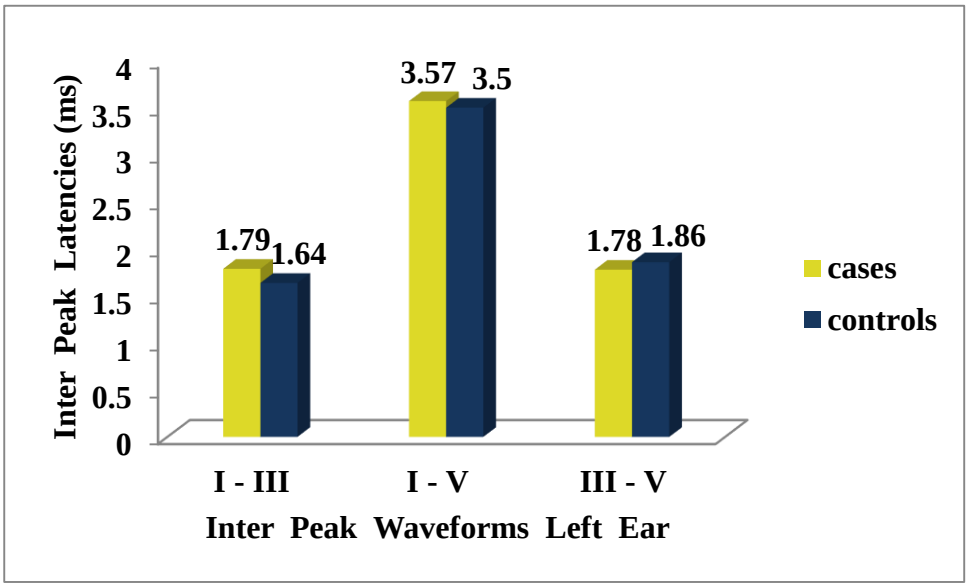


Graph 4 : Absolute wave latencies of left ear compared between vitiligo individuals and control group

Table 6 : Inter Peak Latencies of left ear compared between vitiligo individuals and control group

Inter Peak Waveforms	Inter Peak Latencies (ms)		P value
	Vitiligo individuals n=40 Mean ± SD	Controls n = 40 Mean ± SD	
I – III	1.91 ± 0.16	1.77 ± 0.17	< 0.001**
I – V	3.69 ± 0.15	3.62 ± 0.12	0.06
III – V	1.78 ± 0.19	1.85 ± 0.17	0.07

** P < 0.01 highly significant ; Student independent t - test



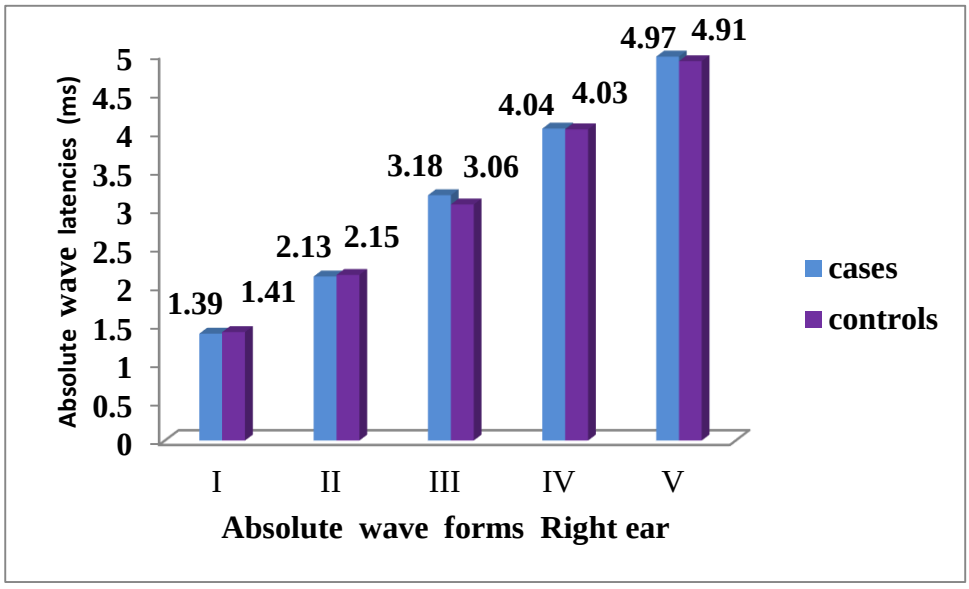
Graph 5

Inter peak latencies of left ear compared between vitiligo individuals and control group

Table 7 : Absolute wave latencies of right ear compared between vitiligo individuals and control group

Absolute wave forms	Absolute wave latencies (ms)		P value
	Vitiligo individuals n=40 Mean ± SD	Controls n = 40 Mean ± SD	
I	1.39 ± 0.06	1.41 ± 0.07	0.19
II	2.13 ± 0.14	2.15 ± 0.13	0.69
III	3.18 ± 0.17	3.06 ± 0.15	0.001**
IV	4.04 ± 0.14	4.03 ± 0.14	0.72
V	4.97 ± 0.13	4.91 ± 0.15	0.09

** P < 0.01 highly significant ; Student independent test

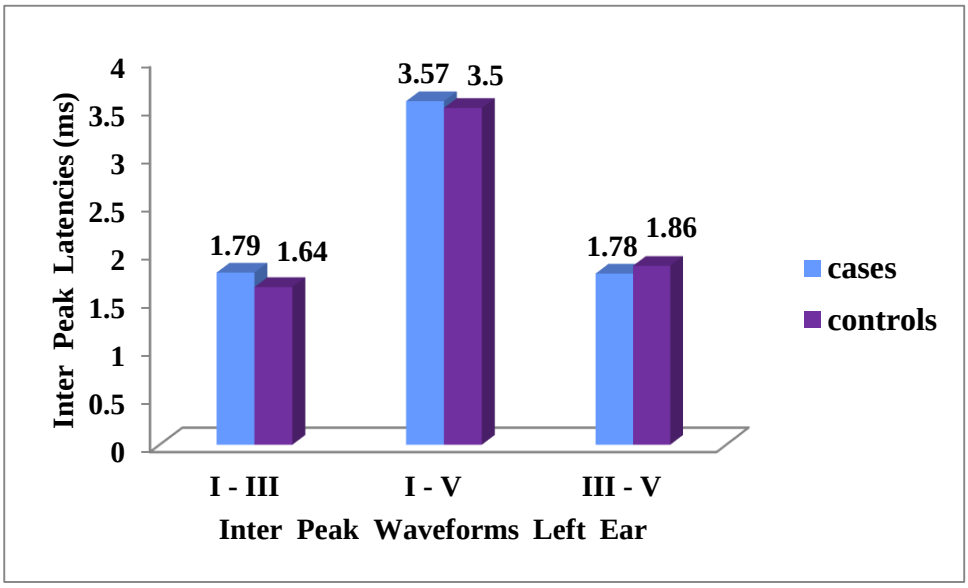


Graph 6 : Absolute wave latencies of right ear compared between vitiligo individuals and control group

Table 8 : Inter Peak Latencies of right ear compared between vitiligo individuals and control group

Inter Peak Waveforms	Inter Peak Latencies (ms)		P value
	Vitiligo individuals n=40 Mean ± SD	Controls n = 40 Mean ± SD	
I – III	1.79 ± 0.17	1.64 ± 0.16	< 0.001**
I – V	3.57 ± 0.16	3.50 ± 0.18	0.06
III – V	1.78 ± 0.21	1.86 ± 0.20	0.117

** P < 0.01 highly significant ; Student independent t - test



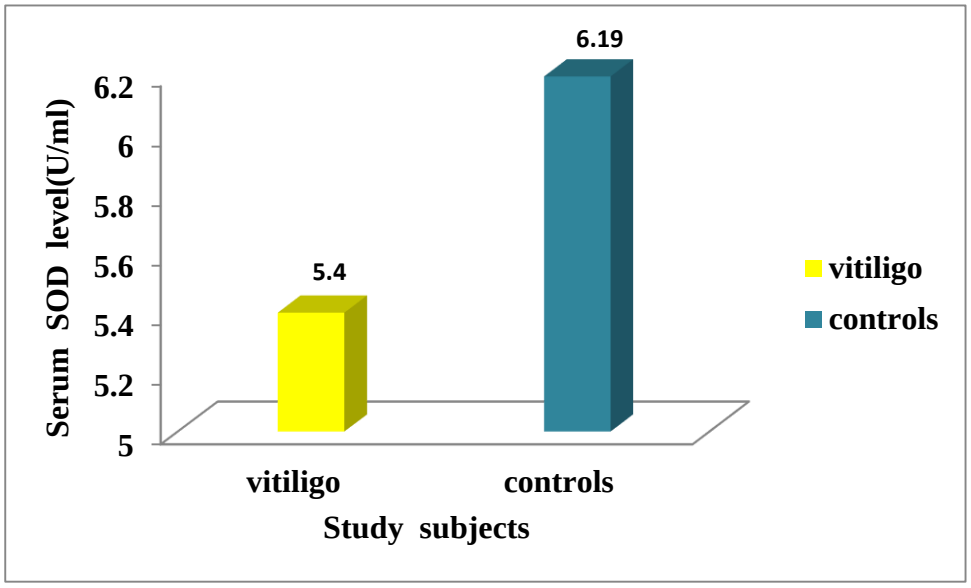
Graph 7

Inter peak latencies of right ear compared between vitiligo individuals and control group

Table 9 : Comparison of serum Superoxide dismutase enzyme levels between vitiligo individuals and control group

Group	SOD Mean ± SD in U/ml	P value
vitiligo individuals	5.40 ± 2.9	0.2
Controls	6.19 ± 3.04	

P > 0.05 not significant ; Student independent t – test



Graph 8

Comparison of serum Superoxide dismutase enzyme levels between vitiligo individuals and control group

6.DISCUSSION

6.1 CHARACTERISTICS OF THE STUDY SUBJECTS

The mean age of vitiligo subjects included in the study was 28.7 ± 6.92 years, who ranged from 15 to 40 years. The mean age of vitiligo subjects in the study by Aydogan K. et al, 2005 was 35.9 ± 12.1 years, in the study by Arican O. et al, 2008, it was 24.9 ± 18.6 years and the mean age was 33.32 years in the study by Sravani P. V. et al, 2009,

The mean duration of vitiligo among the study subjects was 3.57 ± 2.11 years, in the range between 1 to 10 years, whereas the mean duration of vitiligo in the study by Arican O. et al, 2008, was 4.1 ± 7.3 years. Sravani P. V. et al, 2009 observed the mean duration of 11.3 years in his study subjects.

6.2 BRAINSTEM EVOKED RESPONSE AUDIOMETRY

Brainstem Auditory Evoked Potential (BAEP) is the surface recording of the endogenous electrical activity of the underlying brain structure (auditory pathway) resulting from a stimulus bound activity. BAEPs assess the conduction of the impulse through the auditory pathway upto the midbrain. A brief auditory stimulation generates action potentials in the auditory pathway. This technique has been found to be relatively simple, safe and non invasive to implement. The present study

was carried out to record the early changes that occur in the eighth cranial nerve due to vitiligo. The wave latency reflects the duration of evaluation process for an event or a stimulus.

The study in normal subjects showed that BAEP is a reliable method and that latencies and waveforms are constant. This reliability depends on a few important factors maintained during the study, which include stimulus frequency and intensity, position of electrodes, auditory threshold level and laboratory temperature [46].

The functions of melanocytes or melanin in melanocytes are not clearly known. Melanocytes are present in stria vascularis, vestibular organ, endolymphatic sac of the inner ear and auditory receptors or hair cells [2,60]. Melanocytes also play a role in the development of endocochlear potentials and development of the ion and fluid gradient between the endolymph and the perilymph, which are critical for hair-cell survival. Reduced levels and / or reduced activity of pigment cells may cause audiological abnormalities [7,62,63]. It has been reported that melanin has semi conductive properties, responding to acoustic, electrical and phonic stimulation and has the ability to convert energy states into molecular rotation and vibration as well the reverse process [61]. The inner-ear melanin functions as an intracellular calcium buffer and a depot of essential metal ions that control metabolic processes and the activity of various enzymes [60-64]. In fact, a few studies have shown that there were

some abnormalities of BERA reported in vitiligo [10,12,49]. Different results have been reported like decrease in Absolute latency of wave I, increase in absolute latency of wave III, wave V and an increase in Inter Peak Latency I – III in various studies of BERA in vitiligo.

The main parameters studied in BERA are Absolute wave latencies and Inter Peak Latencies. Hence in our study, absolute wave latencies I, II, III, IV and V and inter peak latencies I – III, I – V and III – V between the study group and control group were studied.

6.2.1 Absolute Wave Latencies of left ear:

A significant ($P < 0.05$) increase in the absolute wave latency III of left ear was observed in vitiligo subjects with the mean value of 3.34 ± 0.16 ms, when compared with that of the controls, who had their mean value of 3.27 ± 0.19 ms as depicted in Table 5, Graph 4. Similar results were observed by Aydogan et al, 2005 .

The prolonged Absolute wave latency III may be due to the abnormality in synaptic activity and transmission of action potential from the auditory nerve to the cochlear nucleus and from the cochlear nucleus to the supraoptic nucleus and inferior colliculus. Hence, vitiligo may be associated with delay in synchronization of action potentials in these nuclei.

6.2.2 Absolute Wave Latencies of right ear:

A statistically significant ($P < 0.01$) increase in the Absolute wave latency III of right ear was observed in vitiligo subjects with the mean value of 3.18 ± 0.17 ms when compared with that of the controls who had mean value 3.06 ± 0.15 ms as depicted in Table 7, Graph 6. Similar results were observed by Aydogan et al, 2005 .

The delay in synchronization of action potentials in the auditory pathway could be the explanation for this prolongation in the Absolute wave latency III.

6.2.3 Inter Peak Latencies of left ear:

A statistically significant ($P < 0.01$) increase in the Inter Peak Latency I - III (IPL I – III) of left ear was observed in vitiligo subjects with the mean values of 1.91 ± 0.16 ms, when compared with that of the controls who had 1.77 ± 0.17 ms as depicted in Table 6, Graph 5. Aydogan et al, 2005 observed similar results in their study.

In studies performed by Bassiouny et al, 1998 and Nikifordis et al, 1993, there was prolonged inter peak latency I – III in the vitiligo subjects when compared with that of the controls. The increase in IPL I – III, in their study was due to decrease in Absolute wave I latency, which may be due to the decrease in the number of active melanocytes

in the inner ear. This could have caused the impairment of ionic exchange between perilymph and endolymph.

6.2.4 Inter Peak Latencies of right ear:

A statistically significant ($P < 0.01$) increase in the Inter Peak Latency I - III (IPL I – III) of right ear was observed in vitiligo subjects with the mean values of 1.79 $\pm$ 0.17 ms, when compared with that of the controls who had 1.64 $\pm$ 0.16 ms as depicted in Table 8, Graph 7.

Prolonged IPL I – III was also observed in studies done by Aydogan et al. 2005, Bassiouny et al. 1998, and Nikifordis et al. 1993.

Ozuer et al. 1998, and Shalaby et al. 2006, have observed no significant difference in the Absolute wave latencies and the Inter Peak Latencies in the BERA between the vitiligo subjects and the controls.

The prolonged Inter Peak Latency I – III in both the right and left ear suggests that this may be due to the abnormality in synaptic activity and transmission of action potential from the auditory nerve to the cochlear nucleus and from the cochlear nucleus to the supraoptic nucleus and inferior colliculus. Hence, vitiligo may be associated with delay in synchronization of action potentials in these nuclei and hence in the auditory pathway.

6.3 SUPEROXIDE DISMUTASE ENZYME LEVELS

There was a decrease in the serum level of superoxide dismutase enzyme in the vitiligo subjects, with the mean value 5.40 $\pm$ 2.9 U / ml (Range : 2.6 – 12 U/ml) when compared to that of the controls, with the mean value of 6.19 $\pm$ 3.04 (Range : 2.8 – 11.3 U/ml) as depicted in Table 9, Graph 8. However, this was not statistically significant.

Several studies were done on the various biomarkers such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and nitric oxide (NO) in the vitiligo subjects. Different results were reported about the levels of these biomarkers.

Yildirim et al. 2004, Damma et al. 2009 and Sravani et al. 2009, observed high levels of superoxide dismutase and glutathione peroxidase and low levels of catalase [53,54,55] in the vitiligo subjects when compared with that of the controls.

Koca et al. 2004 and Akrem et al 2009 reported low levels of superoxide dismutase, catalase and glutathione peroxidise in the vitiligo patients when compared with that of the controls [56,57].

Picardo et al. 1994 and Passi et al. 1998 found no difference in blood levels of superoxide dismutase, glutathione peroxidise, lipoperoxidase, vitamin E and ubiquinone between vitiligo subjects and controls [58,59].

Jalel A. et al. 2009 studied oxidative stress in experimental vitiligo mice and reported that the generalised oxidative stress played a role in the pathogenesis of vitiligo. Plasma levels of malondialdehyde were significantly higher ($P < 0.001$) than that of the controls. Superoxide dismutase, Catalase and Glutathione peroxidase levels were significantly lower than that of the controls.

Several studies suggest that the accumulation of free radicals, which are toxic to melanocytes may lead to their destruction. The inherent capacity of cells to withstand the oxidative stress is due to their ability to repair oxidatively modified biomolecules, their antioxidant ability and their capacity to sustain metabolic requirements by deriving energy from alternate pathways.

7. CONCLUSION

From the present study, it can be concluded that there is a conduction delay in the auditory pathway in individuals with vitiligo, as evidenced by abnormality in Brainstem Auditory Evoked Potential latency parameters.

Melanin may play a significant role in the establishment and / or maintenance of the structure and function of the auditory system. It may also modulate the transduction of the auditory stimuli by the inner ear. Melanin containing cells in the inner ear may have a protective function and melanin was reported to play a role in calcium homeostasis of the endolymph.

Brainstem Evoked Response Audiometry (BERA) is a simple and non-invasive procedure which may be done in the vitiligo subjects to provide an early insight about the probable involvement of the eighth cranial nerve and the brainstem auditory pathway. Since the BERA findings were abnormal in clinically asymptomatic vitiligo individuals, this test can be included in the periodical follow up of vitiligo subjects for early detection of subclinical audiological involvement.

The other objective of this study was to evaluate the role of oxidative stress in the pathogenesis of vitiligo, by estimating serum superoxide dismutase enzyme level. Superoxide dismutase enzyme

catalyzes the conversion of superoxide anions to oxygen and hydrogen peroxide. It protects the cells from the toxic effects of superoxide radicals. The accumulation of free radicals which are toxic to the melanocytes, depends on the inherent ability of the cells to withstand the oxidative stress.

There was a decrease in the serum level of superoxide dismutase enzyme in the vitiligo subjects when compared with that of the controls. This confirms the imbalance of the oxidant / anti-oxidant systems in vitiligo. However, further studies on a larger population are needed to substantiate the study results and to stress the importance of antioxidants in reducing the oxidative stress in vitiligo subjects.

8. SUMMARY

The purpose of this study was to detect the subclinical audiological changes in subjects with vitiligo. Since these subjects are usually asymptomatic, the abnormal Brainstem Evoked Response Audiometry (BERA) changes will be of greater clinical significance.

Forty vitiligo subjects and forty normal subjects participated in the study. Brainstem Evoked Response Audiometry was done for them. The serum level of Superoxide dismutase enzyme was also estimated.

The BERA showed increase in the Absolute wave latency III and Inter Peak Latency I – III of both the ears in the vitiligo individuals. These audiological changes in vitiligo individuals confirm that the vitiligo represents a systemic disease with widespread involvement of body melanocytes. Reduced levels or reduced activities of pigment cells or both may be the explanation for audiological abnormalities.

Also, the decreased antioxidant level in vitiligo subjects suggests a state of oxidative stress in them. This could be the probable cause for subclinical audiological changes.

Hence, Brainstem Evoked Response Audiometry can be included in the periodical follow up of vitiligo subjects, to detect early subclinical audiological involvement in them. Further studies are needed to substantiate the importance of the antioxidant therapy in the management of vitiligo in addition to the specific therapies.

9. BIBLIOGRAPHY

1. Sandipan dhar, Pijush dutta, Rajib Malakar. Pigmentary disorders. In: RG vatia, Amit R Valia. IADVL text book of dermatology, vol.1 3rd edn. Mumbai India: Bhalani publisher house; p.749-52.

2. Halder RM, Taliaferro SJ. Vitiligo. In: Wolff K, Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ, eds. Fitzpatrick's Dermatology in General Medicine, 7th Ed. Volume 1. New York: Mc – Graw Hill Companies; 2008. P 616-29.

3. J Clin Biochem (Jan – Mar 2011) 26 (1):78 – 81 DOI 10 1007/3 12291 – 010 – 0045 – 7.

4. Misra UK, Kalito J. Clinical Neurophysiology, 2nd edn. Elsevier publishers; Chapter 2 : Page 11 – 21.

5. Ortonne JP, Bose SK. Vitiligo : where do we stand ? Pigment Cell Res 1993; 6: 61 – 72.

6. Cowan Cl, Jr. Halder RM, Grimes PE. Ocular disturbances in vitiligo. J Am Acad Dermatol 1986;15:17 – 24.

7. Tosti A, Bardazzi F, Tosti G, Monti L. Audiologic abnormalities in cases of vitiligo. J Am Acad Dermatol 1987;17:230 – 3.

8. Orecchia G, Marelli MA, Fresa D, Robiolio L. Audiological disturbances in vitiligo. J Am Acad Dermatol 1989;21:1317 – 8.

9. Escalente – Ugalde C, Poblano A, Montes de oca. No evidence of hearing loss in Patients with vitiligo. Arch Dermatol 1991;127:1240.

10. Nikifordis GC, Tsambaos DG, Karamitsos DS. Abnormalities of the auditory Brainstem response in vitiligo. Scand Audiol 1993;22:97 – 100.

11. Ardic FN, Aktan S, Kara CO, Sanli B. High frequency hearing and reflex latency In patients with pigment disorder. Am J Otolaryngol 1998;19:365 – 9.

12. Ozuer MZ, Sahiner T, Aktan S. Auditory evoked potentials in vitiligo patients. Scand Audiol 1998;27:255 – 8.

13. Sharma L, Bhawan R, Jain RK. Hypoacusis in vitiligo. Indian J Dermatol Venereol Leprol 2004;70:162 – 4.

14. BB, Tarwade YV, Dambre GM, Vitiligo monograph. Pune Gokhale Mediservice Trust, 1989.

15. Betteridge J. What is oxidative stress. Metabolism 2000;49:3 – 8. PMID : 10693912.

16. Bologna J, Pawelek JM. Biology of hypopigmentation. J Am Acad Dermatol 1988;19:217 – 55.

17. Lerner AB. On the etiology of vitiligo and gray hair. Am J Med 1971;51:141 – 7.

18. Gawkrodger DJ, Ormerod AD, Shaw L. Guideline for the diagnosis and Management of Vitiligo. Br J Dermatol 2008;159:1051 – 76.

19. Rook's Textbook of Dermatology, Eighth Edition, Volume 3:58 - 45.

20. Sandipan dhar, Pijush dutta, Rajib Malakar. Pigmentary disorders. In: RG vatia, Amit R Valia. IADVL text book of dermatology, vol.1 3rd edn. Mumbai India: Bhalani publisher house; chapter 25.p.736-60.

21. Theories for the etiology of Vitiligo, chris white. Bulletine for internal circulation for vitiligo surgeon, Tan Research E – publication.

22. Betterle C, Peserico A, Bersani G. Vitiligo and autoimmune polyendocrine deficiencies with autoantibodies to melanin producing cells. Arch Dermatol 1979;115:364.

23. Woolfson H, Finn OA, Mackie RM. Serum anti – tumour antibodies and auto- antibodies in vitiligo. Br J Dermatol 1975;92:395 – 400.

24. Naughton GK, Eisinger M, Bystryn JC. Detection of antibodies to melanocytes in Vitiligo by specific immunoprecipitation. J Invest Dermatol 1983;81:540 – 2.

25. Naughton GK, Reggiardo D, Bystryn JC. Correlation between vitiligo antibodies and extent of depigmentation in vitiligo. J Am Acad Dermatol 1986;15:978- 81.

26. Cui J, Arita Y, Bystryn JC. Cytolytic antibodies to melanocytes in vitiligo. J Invest Dermatol 1993;100:812 – 5.

27. Ghoneum M, Grimes E, Gill G. T cell profiles in vitiligo. J Am Dermatol 1987; 17:600 – 5.

28. Grimes PE, Ghoneum M, Stockton T. T – cell profiles in vitiligo. J Am Acad Dermatol 1986;14:196 – 201.

29. Mozzanica N, Frigerio U, Finzi AF. T cell subpopulations in vitiligo:a chronologic study. J Am Acad Dermatol 1990;22:223 – 30.

30. Al Abadie MSK, Senior HJ, Bleehen SS, Gawkrodger DJ. Neuropeptide and neuronal marker studies in vitiligo. Br J Dermatol 1994;131:160 – 5.

31. Cucchi ML, Frattini P, Santagostino G. Higher plasma catecholamine and metabolite levels in the early phase of nonsegmental vitiligo. Pigment Cell Res 2000;13:28 – 32.

32. Hara M, Toyoda M, Yaar M. Innervation of melanocytes in human skin. J Exp Med 1996;184:1385 – 95.

33. Bleehen SS, Pathak MA, Hori Y, Fitzpatrick TB. Depigmentation of skin with 4 –isopropylcatechol, mercaptoamines and other compounds. J Invest Dermatol 1968 ;50 : 103 – 17.

34. Peus D, Vasa RA, Meves A, Pott M, Beyerle A. H_2O_2 is an important mediator of UVB induced by EGF receptor phosphorylation in cultured melanocytes causing damage to biomolecules. J Invest Dermatol 1998;110: 966 – 71.

35. Dell'Anna ML, Maresca V, Briganti S. Mitochondrial impairment in peripheral mononuclear cells during the active phase of vitiligo. J Invest Dermatol 2001;117: 808–13.

36. Mohr J. Vitiligo in a pair of monovular twins. Acta Genet 195;2:252 – 5.

37. Nishimura EK, Jordan SA, Oshima H, Yoshida H, Osawa M, Moriyama M. Dominant role of the niche in melanocyte stem cell fate determination. Nature 2002;416:854 – 60.

38. Le poole IC, Das PK, van den Wijngaard RM. Review of etiopathomechanism of vitiligo:a convergence theory. Exp Dermatol 1993;2:145–53.

39. Huggins RH, Schwartz RA, Krysicka Janniger C. Vitiligo. Acta Dermatoven APA 2005;Vol 14:No 4.

40. Nordlund JJ, Taylor N, Albert DM. The presence of vitiligo and poliosis in patients with uveitis. J Am Acad Dermatol 1981;4:528 – 36.

41. Cowman CL, Halder RM, Grimes PE. Ocular disturbances in vitiligo. J Am Acad Dermatol 1986;15:17 – 24.

42. Tosti A, Bardazzi F, Tosti G. Audiologic abnormalities in cases of vitiligo. J Am Acad Dermatol 1987;17:230 – 3.

43. Jean Paul ortonne. Vitiligo and other disorders of hypopigmentation, 2nd edition dermatology edited by Jean L – Bolognia, Joseph L Jorizzo, Ronald P Rapini.

44. Anju Jain, Jyoti Mal, Vibhu Mehndiratta, Ram chander, Surajeet Kumar Patra. Ind J Clin Biochem (Jan – Mar 2011) 26 (1) : 78 – 81 DOI 10 . 1007 / S 12291 – 010 – 0045 – 7.

45. Colin Binnie, Ray Cooper, Francois Manguiere, John Osselton, Pamela Prior, Brian Tedman. Clinical Neurophysiology. Volume Revised Enlarged Edition, Edited by : Elsevier. Part 3 : Page 353 – 615.

46. Michael J. Aminoff Electro diagnosis in Clinical neurology. Fifth edition. Elsevier Chap 23: Page 490–2.

47. Mosby, Members of Mayo Clinic, Mayo Clinic Examinations in Neurology, 7th Edition, Editors David O, Wiebers Allan JD, Dale Emre Kokmen Jerry W. Swanson Chapter 15:Page 449–54.

48. Bassiouny A, Farid S, EI Khousht M. Hearing abnormalities in vitiligo. Egypt J Otolaryngol January 1998;15.1:51 – 60.

49. Aydogan K, Turan OF, Onart S, Karadogan SK, Tunali S. (2005): Audiological abnormalities in patients with vitiligo. Experimental dermatology DOI:10:1111 / j.1365 – 2230. 2005 . 02004.x.

50. Mamours El-Sayed Shalaby, Gehan A, El-Zarea, Ahmed I Nassar. Auditory Function In Vitiligo Patients. Egypt Dermatol Online Journal Vol. 2 (1):7, June, 2006.

51. http : blog . target health . com.

52. Karaca S. Guder H. Dermatolojide antioksidan system. Turk J Dermatol 2009;3: 32 – 39.

53. Yildrium M, Baysal V, Inaloz HS, Can M. The role of oxidants and antioxidants in generalized vitiligo at tissue level. JEADV 2004, 18:683–6.PMID:15482295.

54. Dammak I, Boudaya S, Abdallah FB, Turki H, Attia H, Hentati B. Antioxidant enzymes and lipid peroxidation at tissue level in patients with stable and active vitiligo. Int J Dermatol 2009,48:476 –480. PMID : 19416376.

55. Sravani PV, Babu NK, Gopal GRR, Rao AR, Moorthy B, Rao TR . Determination of oxidative stress in vitiligo by measuring superoxide dismutase and catalase levels in vitiliginous and non-vitiliginous skin. Indian J Dermatol Venereol 2009, 75:268 – 271. PMID:19439879.

56. Koca R, Armutcu F, Atinyazar HC, Gurel A. Oxidant – antioxidant enzymes and lipid peroxidation in generalised vitiligo. Clin Exp Dermatol 2004;29:406 – 409. PMID : 15245542.

57. Jalel A, Yassine M, Hamdaoui MH. Oxidative stress in experimental vitiligo C57BL / 6 mice. Indian J Dermatol 2009, 54:221 – 324. PMID : 20161850.

58. Picardo M, Passi S, Morrone A, Grandinetti M, Di Carlo A, Ippolito F. Anti- oxidant status in the blood of patients with active vitiligo. Pigment cell Res 1994,7:110 – 115. PMID : 8066016.

59. Passi S, Grandinette M, Maggio F, Stancato A, De Luca C. Experimental oxidative stress in vitiligo. Pigment cell Res 1998, 11:81 – 85. PMID : 9585244.

60. Barrenas ML. Axelsson A. The development of melanin in the stria vascularis of the ger bil. Acta Otolaryngol 1992:112: 50 – 8.

61. Mc Ginness J. Corry P. Proctor P. Amorphous semiconductor switching in melanins. Science 1974:183:853 – 5.

62. Steel KP. Barkway C. Another role for melanocytes:their importance for normal stria vascularis development in mammalian inner ear. Development 1989;107: 453 – 63.

63. Tachibana M. Sound needs sound melanocytes to be heard. Pigment cell Res 1999:12:344 – 54.

64. Barrenas ML. Lindgren F. The influence of inner ear melanin on susceptibility to YYS in human. Scand Audiol 1990:19:97-102.

65. Zeeca L. Shima T. Stroppolo A et al. H.M. Interaction of neuromelanin and iron in substantia nigra and other areas in human brain. Neuroscience 1996:73: 407 – 15.

66. Malstrom, B., Andreasson, L., and Reinhammer, B. In The Enzymes . Boyer, P., editor. XII B, Academic Press, New York, 533 (1975).

67. Sandstrom, J., Nilsson, P., Karllson, K., et al.10 – Fold increase in human plasma extracellular superoxide dismutase content caused by a

mutation in heparin binding domain. J. Biol. Chem. 269 (29), 19163 – 19166 (1994).

68. Marklund, S., Distribution of CuZn superoxide dismutase in human tissues and extracellular fluids. Acta Physiol. Scand. Suppl.492, 19 – 23(1980).

69. Sun, E., Xu, H., Liu, Q., et al. The mechanism for the effect of selenium supplementation on immunity. Biol. Trace Elem. Res. 48 (3), 231–238 (1995).

70. Beyer W, Imlay J, Fredovich I. Superoxide dismutase. Progr Nucl Acid Res Mole Biol 1991;40:221–53.

71. Stallings W, Pattridge K, Strong R, Ludwig M. Manganese and iron superoxide dismutases are structural homologs. J Biol Chem 1984 ; 259:10695–9.

72. Kovacs SO. Vitiligo. J Am Acad Dermatol 1998 ; 38 : 647–66.

73. Maresca V, Roccella F, Camera E, Del Porto G, Passi S, et al. Increased sensitivity to peroxidative agents as a possible pathogenic factor of melanocyte damage in vitiligo. J Invest Dermatol 1997;109:310–3.

74. Knight JA. Diseases related to oxygen derived free radicals. Ann Clin Lab 1995; 25:111–21.

(ii) ஒப்புதல் படிவம்

திரு/திருமதி/செல்வன்/செல்வி/_____
ஆகிய நான் டாக்டர்.xxxxxxxxxxxx பட்டமேற்படிப்புமாணவி, உடல் இயங்கியல் துறை, ஸ்டான்லி மருத்துவக் கல்லூரி, மருத்துவ- மனையில் நடத்தும் ஆராய்ச்சியில் யாருடைய வற்புறுத்தலும் இன்றி என்னுடைய முழுசம்மதத்துடன் பங்கேற்க சம்மதம் தெரிவிக்கிறேன். இந்த ஆராய்ச்சி, தோல் நிறமிகளின் குறை- பாட்டால், செவித்திறன் நரம்புகளில் ஏற்படும் விளைவுகளை அறிந்து கொள்ள உதவியாக இருக்கும் என்பதை நான் அறிந்து கொண்டேன். ஆராய்ச்சியின் செயல்பாடுகளை ஆராய்ச்சியாளர் மூலம் அறிந்து கொண்டேன். இந்த ஆராய்ச்சியில் எந்தவித மருந்துகளோ ஊசிகளோ அளிக்கப்படமாட்டாது எனவும் நான் இந்த ஆராய்ச்சியில் இருந்து எந்தவித முன்னறிவிப்புமின்றி விலகிக்கொள்ள எனக்கு உரிமை உண்டு எனவும் இந்த ஆராய்ச்சியின் ஏடுகள் இரகசியமாக வைக்கப்படும் என்பதையும் நான் அறிவேன்.

தேதி :

ஆய்வில் பங்கேற்பவர் கையொப்பம்

அல்லது பெருவிரல் பதிவு

(ii) CONSENT FORM

I Mr/Mrs/Ms _____

understand that Dr.xxxxxxxxxxxx a postgraduate student in Stanley Medical college and Hospital, Chennai is doing the study on Vitiligo individuals and control group. I have been made to understand that the test will assess the functioning of my ears. The test is simple, involve recording of nerve conduction in the ears. They do not involve injections or taking any medicines and are risk free. I have been familiarised with the testing procedures. I am participating in this study willingly. I have not been forced to do so. I have also been told clearly that I could withdraw from this study without any prejudice.

Date :

Signature of the participant / Thumb impression

(iii) PROFORMA

Proforma for the study subject Date :

1. Sl. No. :

2. I.D. No.(given by investigator) : Address & Contact No.

3. Name : Ht.

4. Age : Wt.

5. Gender : Religion :

6. Dermatology Dept. No. / Master Health checkup No. :

7. Occupation : Not working/ Housewife/ labourer – what type of

Work / Professionals/ Others (specify)

8. Family History : Vitiligo/ Endocrine disorder/ Others

Parent / Siblings affected

9. Personal History : Diabetes mellitus/ Thyroid disorder

10. Past History : Head injury/ Chronic noise exposure

11. Treatment History : Oral drugs / Parenteral

Topical agents

12. Depigmented skin lesions : Duration

Site of distribution

Focal/ Segmental/ Vulgaris/ Acrofacial

13. Hearing impairment : Yes/ No

14. General Examination : Alert/ Anaemia/ Jaundice/ Cyanosis/ Clubbing

15. Vital signs : Temp.: / Pulse rate : / Blood pressure

16. Systemic Examination : Cardiovascular system :

Respiratory system :

Abdomen :

Central Nervous system :

17. Local Examination : Depigmented skin lesions

18. Ear, Nose , Throat examination :

Audiological Examination :

1. Examination of the external auditory meatus

2. Examination of the tympanic membrane

3. Tuning fork tests

a) Rinnie test:

b) Weber test:

4. Pure tone audiometry

a) Right ear:

b) Left ear:

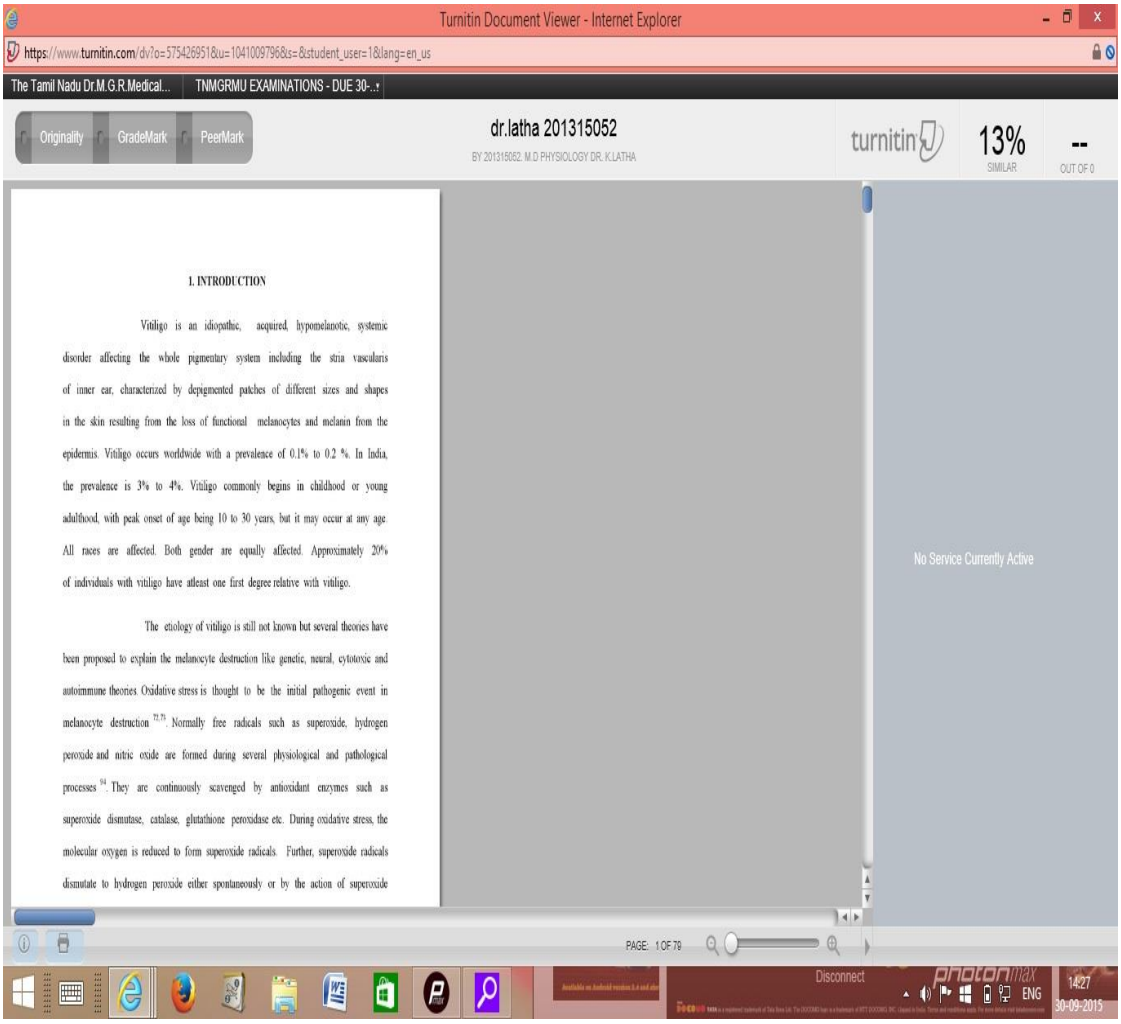
5. Brainstem Auditory Evoked Response:

Variables (ms)	Left Ear	Right Ear
Wave Latency I		
Wave Latency II		
Wave Latency III		
Wave Latency IV		
Wave Latency V		
Inter Peak Wave Latency I-III		

Inter Peak Wave Latency I-V		
Inter Peak Wave Latency III-V		

Cases No.	Age	sex	Types	Height	Weight	BMI	Right Ear								Left Ear								SOD
Vitiligo	yrs		F,AF,S	cm	kg		RL1	RL2	RL3	RL4	RL5	RIPL 1-5	RIPL 1-3	RIPL 3-5	LL1	LL2	LL3	LL4	LL5	LIPL 1-5	LIPL 1-3	LIPL 3-5	U/ml
1	22	M	AF	162	65	24.8	1.42	2.08	3.4	4.1	4.9	3.38	1.78	1.5	1.52	2.35	3.4	4.22	5.03	3.51	1.88	1.63	7
2	31	F	V	150	65	28.9	1.5	2.5	3.2	4	4.8	3.28	1.52	1.6	1.5	2.22	3.3	4.35	5.1	3.6	1.8	1.8	4.5
3	34	M	V	161	70	27	1.45	2	3.1	4.1	4.62	3.33	1.57	1.52	1.35	2.2	3.3	4.28	5.3	3.95	1.95	2	3.5
4	16	F	S	152	48	20.8	1.47	2.28	3.4	4	5.08	3.56	1.73	1.68	1.55	2.62	3.55	4.3	5.2	3.65	2	1.65	4.2
5	24	M	AF	163	62	23.5	1.4	2.52	3.2	4.28	4.9	3.02	1.61	1.7	1.46	2.68	3.3	4.3	4.9	3.44	1.84	1.6	6
6	18	M	S	160	68	26.6	1.48	2.1	3.1	4.1	4.6	3.02	1.5	1.5	1.4	2.1	3.4	4.3	4.9	3.5	2	1.5	3.5
7	19	F	F	151	45	19.7	1.4	2.08	3.45	4.02	4.85	3.42	1.92	1.4	1.4	2.4	3.65	4.3	5.28	3.86	2.23	1.63	6.5
8	16	M	S	165	63	23.1	1.5	2	3.2	3.82	4.87	3.3	1.5	1.67	1.4	2.22	3.32	3.98	5	3.6	1.92	1.68	5
9	21	M	AF	167	68	24.4	1.3	2.22	3.3	4.38	4.78	3.7	1.9	1.48	1.48	2.42	3.68	4.5	5.28	3.8	2.2	1.6	2.6
10	32	F	V	152	60	26	1.4	2.17	3.5	4	4.95	3.8	2	1.45	1.58	2.32	3.8	4.63	5.3	3.72	2.22	1.5	6.4
11	23	F	S	152	65	28.1	1.3	2.48	3.38	4.2	5	3.6	2.1	1.62	1.38	2.45	3.65	4.1	5	3.62	2.27	1.35	3.3
12	40	F	V	150	65	28.9	1.4	2.15	3.02	4.03	4.9	3.6	1.6	1.88	1.45	2.25	3.25	4.1	5	3.55	1.8	1.75	4.9
13	20	M	S	165	70	25.7	1.5	1.9	3.1	4	5	3.4	1.7	1.9	1.42	2.55	3.4	4.37	5.1	3.68	1.98	1.7	12
14	31	F	V	161	68	26.2	1.33	2.08	3.18	4.22	4.97	3.67	1.77	1.79	1.35	2.38	3.32	4.6	5.3	3.95	1.97	1.98	5.6
15	40	F	V	158	65	26	1.3	2.22	3.28	3.92	4.98	3.7	1.7	1.7	1.4	2.22	3.42	4.3	5.2	3.8	2.02	1.78	3.7
16	23	F	AF	157	58	23.5	1.5	2.32	3.5	4.3	5	3.6	1.8	1.5	1.48	2.52	3.48	4.5	5	3.52	2	1.52	6.7
17	31	F	V	152	65	28.1	1.47	2.2	3.2	4.1	5	3.53	1.53	1.8	1.6	2.5	3.2	4.2	5	3.4	1.6	1.8	3.4
18	34	F	F	156	64	26.3	1.4	2	3.3	4.4	5	3.6	1.8	1.7	1.3	2.2	3.4	4.5	5	3.7	2.1	1.6	5
19	34	F	V	155	70	29.1	1.5	2.2	3.4	4.2	5.1	3.4	1.7	1.7	1.3	2.5	3.3	4.1	5	3.7	2	1.7	3.5
20	36	F	V	153	65	27.8	1.5	2.2	3.3	4	4.8	3.2	1.7	1.5	1.5	2.4	3.3	4.2	5	3.5	1.8	1.7	4
21	28	M	AF	168	75	26.6	1.4	2	3.2	3.8	4.9	3.3	1.6	1.7	1.6	2.5	3.3	4	5.1	3.5	1.7	1.8	4.3
22	40	F	V	165	66	24.2	1.38	2	2.8	3.8	4.9	3.42	1.52	2.1	1.3	2.3	3.2	4	5	3.7	1.9	1.8	4
23	37	M	V	175	70	22.9	1.3	2.2	3.3	4	5	3.6	1.7	1.7	1.5	2.3	3.3	4	5	3.5	1.8	1.7	14
24	30	M	V	168	75	26.6	1.4	2.1	3	4	5	3.4	1.5	2	1.43	2.2	3.3	4	5.1	3.67	1.87	1.8	8
25	30	M	F	165	66	24.2	1.5	2.1	3.2	4	4.9	3.5	1.5	1.7	1.43	2.2	3.4	4.2	5.3	3.87	1.97	1.9	6.9
26	29	M	AF	165	60	22	1.3	2.1	3.1	4	5.1	3.7	1.7	2	1.4	2.1	3.1	4.1	5.3	3.9	1.7	2.2	5
27	36	F	V	155	52	21.6	1.4	2	3.2	3.9	5	3.5	1.7	1.8	1.5	2.2	3.3	4	5	3.5	1.8	1.7	3.9
28	28	M	V	165	66	24.2	1.5	2	3.1	4	5	3.4	1.5	1.9	1.4	2.4	3	3.9	5.1	3.7	1.6	2.1	3.6
29	23	F	AF	153	65	27.8	1.5	2	3	4	5	3.5	1.4	2	1.4	2	3.1	4	5.1	3.7	1.7	2	3.6
30	22	F	S	155	52	21.6	1.4	2.1	2.8	3.8	4.8	3.5	1.3	2	1.4	2	3.1	4.1	5.2	3.8	1.7	2.1	3
31	19	M	S	168	60	21.3	1.46	2	3	4	5.1	3.54	1.44	2.1	1.4	2.2	3.3	3.9	5.2	3.8	1.9	1.9	2.7
32	27	F	AF	158	70	28	1.48	2	3	4	5.1	3.62	1.52	2.1	1.3	2.1	3.2	4.2	5.1	3.8	1.9	1.9	6.1
33	33	F	V	153	65	27.8	1.3	2.1	3	3.9	5.1	3.7	1.6	2.1	1.3	2	3.2	4.1	5.2	3.9	1.9	2	11.7
34	36	F	V	155	70	29.1	1.5	2.2	3.2	4	5.1	3.5	1.6	1.9	1.5	2.2	3.4	4.2	5.1	3.6	1.9	1.7	4.6
35	31	M	S	173	80	26.7	1.4	2.2	3.2	4.1	5.1	3.7	1.7	1.9	1.36	2.5	3.3	4.3	5.3	3.94	1.94	2	4.9
36	26	F	F	152	52	22.5	1.3	2	3.2	4	5.2	3.7	1.6	2	1.36	2.1	3.4	4.2	5	3.64	2.04	1.6	11
37	40	M	V	172	74	25	1.3	2.2	3.2	4.1	5	3.6	1.7	1.8	1.4	2.2	3.4	4.5	5	3.6	2	1.6	3.6
38	29	F	AF	158	70	28	1.4	2.2	3.1	4	5.1	3.6	1.6	2	1.42	2.3	3.4	4.2	5.2	3.78	1.98	1.8	3.9
39	32	F	V	152	60	26	1.38	2	3	4	5.1	3.62	1.52	2.1	1.38	2	3.2	4.1	5.3	3.92	1.82	2.1	3.1
40	27	F	AF	154	60	25.3	1.4	2.1	3.2	4	5.1	3.5	1.6	1.9	1.42	2.2	3.2	4	5.1	3.68	1.78	1.9	4.1

Control	Age	Sex	Heigh	Weigh	BMI	Right Ear								Left Ea								SOD)
No.	yrs		cm	kg		RL1	RL2	RL3	RL4	RL5	RIPL 1-	RIPL 1-3	RIPL 3-5	LL1	LL2	LL3	LL4	LL5	LIPL 1-5	LIPL 1-3	LIPL 3-5	U/ml
1	29	M	172	70	23.7	1.42	2.05	3.2	4	4.8	3.38	1.78	1.6	1.55	2.2	3.38	4.35	5.18	3.63	1.83	1.8	4
2	35	F	163	58	21.8	1.5	2.22	3.02	4	4.78	3.28	1.52	1.76	1.52	2.3	3.4	4.2	5.2	3.68	1.88	1.8	4
3	29	F	169	52	18.2	1.45	1.98	3.02	4.05	4.78	3.33	1.57	1.76	1.45	2.1	3.22	4.4	5.3	3.85	1.77	2.08	6.2
4	29	M	168	64	22.7	1.47	2.2	3.2	4	5.03	3.56	1.73	1.83	1.6	2.4	3.45	4.3	5.32	3.72	1.85	1.87	5
5	33	F	168	58	20.5	1.4	2.48	3.01	4.03	4.42	3.02	1.61	1.41	1.48	2.5	3.3	4.2	4.88	3.4	1.82	1.58	5
6	27	F	164	70	26	1.48	2	2.98	4.03	4.5	3.02	1.5	1.52	1.5	2.1	3.3	4.28	4.9	3.4	1.8	1.6	9
7	40	F	158	65	26	1.4	2.17	3.32	3.92	4.82	3.42	1.92	1.5	1.42	2.35	3.6	4.4	5.2	3.78	2.18	1.6	8
8	27	F	154	52	21.9	1.5	2	3	3.9	4.8	3.3	1.5	1.8	1.4	2.3	3.2	4.1	5	3.6	1.8	1.8	3.5
9	25	F	156	64	26.3	1.3	2.2	3.2	4.2	5	3.7	1.9	1.8	1.45	2.3	3.6	4.5	5.2	3.75	2.15	1.6	3.8
10	36	M	173	70	23.4	1.4	2.2	3.4	4.2	5.2	3.8	2	1.8	1.6	2.4	3.7	4.6	5.4	3.8	2.1	1.7	4.2
11	29	F	163	62	23.5	1.3	2.5	3.4	4.1	4.9	3.6	2.1	1.5	1.4	2.4	3.6	4.2	5	3.6	2.2	1.4	4.2
12	32	F	165	63	23.1	1.4	2.2	3	4.1	5	3.6	1.6	2	1.5	2.3	3.3	4.2	5.2	3.7	1.8	1.9	4.3
13	40	M	175	70	22.9	1.5	2.1	3.2	4.1	4.9	3.4	1.7	1.7	1.5	2.2	3.3	4.2	5	3.5	1.8	1.7	3.6
14	37	M	168	75	26.6	1.33	2.1	3.1	4.2	5	3.67	1.77	1.9	1.4	2.2	3.2	4.5	5.2	3.8	1.9	1.9	4.9
15	40	M	172	80	27	1.3	2	3	4.1	5	3.7	1.7	2	1.4	2.2	3.2	4.3	5.1	3.7	1.8	1.9	9
16	26	F	161	68	26.2	1.5	2.4	3.3	4.2	5.1	3.6	1.8	1.8	1.5	2.6	3.4	4.3	5.2	3.7	1.9	1.8	5.8
17	18	F	152	54	23.4	1.47	2.1	3	4.2	5	3.53	1.53	2	1.6	2.5	3.4	4.3	5.1	3.5	1.8	1.7	6.9
18	40	F	150	65	28.9	1.4	2.2	3.2	4.5	5	3.6	1.8	1.8	1.47	2.4	3.3	4.4	5.1	3.63	1.83	1.8	10.7
19	34	M	169	80	28	1.5	2.4	3.2	4	4.9	3.4	1.7	1.7	1.48	2.6	3.4	4.3	5.2	3.72	1.92	1.8	6.4
20	20	M	168	58	20.5	1.5	2.1	3.2	4	4.7	3.2	1.7	1.5	1.6	2.4	3.2	4.3	5	3.4	1.6	1.8	4.2
21	17	F	154	52	21.9	1.4	2.1	3	3.7	4.7	3.3	1.6	1.7	1.4	2.2	3	3.9	5	3.6	1.6	2	5.5
22	19	F	153	50	21.4	1.38	2.1	2.9	3.8	4.8	3.42	1.52	1.9	1.39	2.2	3.1	4	4.8	3.41	1.71	1.7	8.8
23	24	M	168	75	26.6	1.3	2.2	3	4	4.9	3.6	1.7	1.9	1.4	2.3	3.2	4.1	4.9	3.5	1.8	1.7	6.4
24	29	M	172	74	25	1.4	2	2.9	3.9	4.8	3.4	1.5	1.9	1.41	2.1	3	3.9	5	3.59	1.59	2	7
25	33	M	168	60	21.3	1.5	2.2	3	4	5	3.5	1.5	2	1.42	2.1	3.2	4.1	5.1	3.68	1.78	1.9	11.3
26	23	F	155	52	21.6	1.3	2.1	3	4.1	5	3.7	1.7	2	1.39	2.2	3	4.2	5.1	3.71	1.61	2.1	14
27	18	M	165	60	22	1.4	2.2	3.1	4	4.9	3.5	1.7	1.8	1.43	2.3	3.2	4.1	5	3.57	1.77	1.8	4.6
28	16	M	158	55	22	1.5	2	3	3.9	4.9	3.4	1.5	1.9	1.5	2	3	4	5	3.5	1.5	2	5.7
29	21	M	160	52	20.3	1.5	2.1	2.9	4	5	3.5	1.4	2.1	1.51	2.1	3.2	4.1	5.2	3.69	1.69	2	7
30	28	F	150	58	25.8	1.4	2	2.7	4	4.9	3.5	1.3	2.2	1.46	2.1	2.9	4	5	3.54	1.44	2.1	4.9
31	20	M	168	75	26.6	1.46	2	2.9	3.9	5	3.54	1.44	2.1	1.48	2.1	3	4	5	3.52	1.52	2	7
32	28	M	165	66	24.2	1.48	2.1	3	4.1	5.1	3.62	1.52	2.1	1.5	2.2	3.2	4.2	5.2	3.7	1.7	2	15.6
33	34	F	160	75	29.3	1.3	2	2.9	3.8	5	3.7	1.6	2.1	1.4	2	3.1	4	5.1	3.7	1.7	2	5.6
34	31	M	161	70	27	1.5	2.2	3.1	4.1	5	3.5	1.6	1.9	1.5	2.3	3.2	4.1	5.1	3.6	1.7	1.9	6.6
35	36	F	156	64	26.3	1.4	2.1	3.1	4	5.1	3.7	1.7	2	1.4	2.2	3.1	4.1	5.2	3.8	1.7	2.1	5.8
36	34	F	154	52	21.9	1.3	2	2.9	4	5	3.7	1.6	2.1	1.39	2.1	3	4	4.9	3.51	1.61	1.9	3.3
37	23	F	158	70	28	1.3	2.2	3	4	4.9	3.6	1.7	1.9	1.4	2.4	3.2	4.1	5	3.6	1.8	1.8	3
38	18	F	155	52	21.6	1.4	2.3	3	4.1	5	3.6	1.6	2	1.4	2.5	3.3	4.2	5.1	3.7	1.6	1.8	2.8
39	21	F	158	55	22	1.38	2.1	2.9	4	5	3.62	1.52	2.1	1.4	2.2	3	4	5.2	3.8	1.6	2.2	3.9
40	39	F	158	70	28	1.4	2.2	3	3.9	4.9	3.5	1.6	1.9	1.42	2.3	3.1	4	5	3.58	1.68	1.9	4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1. INTRODUCTION
Vitiligo is an idiopathic, acquired, hypomelanotic, systemic disorder affecting the whole pigmentary system including the stria vascularis of inner ear, characterized by depigmented patches of different sizes and shapes in the skin resulting from the loss of functional melanocytes and melanin from the epidermis. Vitiligo occurs worldwide with a prevalence of 0.1% to 0.2 %. In India, the prevalence is 3% to 4%. Vitiligo commonly begins in childhood or young adulthood, with peak onset of age being 10 to 30 years, but it may occur at any age. All races are affected. Both gender are equally affected. Approximately 20% of individuals with vitiligo have atleast one first degree relative with vitiligo.
The etiology of vitiligo is still not known but several theories have been proposed to explain the melanocyte destruction like genetic, neural, cytotoxic and autoimmune theories Oxidative stress is thought to be the initial pathogenic event in melanocyte destruction [73,75]. Normally free radicals such as superoxide, hydrogen peroxide and nitric oxide are formed during several physiological and pathological processes [94]. They are continuously scavenged by antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase etc. During oxidative stress, the molecular oxygen is reduced to form superoxide radicals. Further, superoxide radicals dismutate to hydrogen peroxide either spontaneously or by the action of superoxide
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